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Effect of smoking upon the vascular beds in skin and muscle, as determined by plethysmographic studies in man. DAVID I. ABRAMSON, HERMAN ZAZEELA (by invitation) and B. S. OPPENHEIMER (by invitation). Medical Service, Mt. Sinai Hospital, New York City, and The May Institute for Medical Research of the Jewish Hospital, Cincinnati, O.

It has been fairly well established that smoking causes vasoconstriction of blood vessels in the skin, but little evidence has been presented as to the response of the vessels in the muscles of the extremities. This latter subject was therefore investigated by means of plethysmographic studies of the forearm, which is composed of muscle to the extent of approximately 85 per cent of its volume. It was found that in no instance was there a decrease in blood flow through this portion of the upper extremity during the period of smoking. That this was not due to a lack of response to the procedure on the part of the subject was readily proved by the presence of a simultaneous and significant decrease in blood flow through the hand (which is composed of skin to the extent of approximately 50 per cent of its volume) and through the forearm combined with the hand. Alterations in flow through the foot and adjoining portion of the leg were generally of smaller magnitude than those in the hand.

By exposing the extremities in the plethysmographs to various water-bath and room temperatures, it was found that the blood vessels in the skin do not respond maximally to smoking when they are either abnormally dilated or excessively constricted. Evidence was also obtained to show that during the period of smoking a continuous state of vasoconstriction exists.

It appears, therefore, that the generally quoted statement that smoking causes a decrease in peripheral circulation should be modified so as to indicate that the vasoconstriction probably takes place only in the blood vessels of the skin and not in those of voluntary muscle. Such a view is in accord with the hypothesis that the blood vessels in these two sites respond differently to most physiological and chemical stimuli.

Protein intake and protein distribution in the organs and tissues of the body.

T. ADDIS. Medical Department, Stanford University Medical School, San Francisco, Calif.

The quantities of protein in the whole body and in the liver, heart, kidneys, alimentary tract, uterus, carcass and in the serum and clot of the

drawn blood were determined in a group of 20 rats whose average body weight was 150 grams. The quantities of protein in these organs and tissues were also determined in 4 groups with an average body weight of 150 grams on the day they were first given food that contained 11 per cent, 16 per cent, 27 per cent and 43 per cent of protein. These determinations were made after 18 days on the above diets. On the assumption that all 5 groups were identical when their body weight was 150 grams the quantities of protein laid down in the various organs and tissues were determined by subtraction of the original from the final values, and related to the quantities of protein consumed. It was found that increase in protein consumption had a different effect on each of the organs and tissues examined, and that the total effect on the protein content of the body was the sum of effects that were sometimes qualitatively as well as quantitatively diverse.

*The signs of acute anoxia in man.*¹ FREDERICK A. D. ALEXANDER (by invitation) and H. E. HIMWICH. Departments of Anesthesia, Physiology and Pharmacology, Albany Medical College, Union University, Albany, N. Y.

Respiratory, cardio-circulatory, neuromuscular and other changes were observed during more than two hundred bouts of acute oxygen want induced in ten patients (for the alleviation of schizophrenia). Anoxemia was produced by having the patient breathe an atmosphere in which the percentage of nitrogen was rapidly increased at the expense of oxygen, without accumulation of carbon dioxide. The bouts lasted 3-4 minutes and were abruptly terminated by inflating the patient's lungs with pure oxygen. The arterial blood oxygen content was reduced to about 2 volumes per cent. Although there was some variation in the response of different individuals and in the same individuals on different days the signs were largely consistent in their chronological appearance.

Respiratory stimulation was the first sign to appear and became progressively greater until extensor muscle spasms occurred when it was still augmented but became arrhythmic. Respiration was sometimes complicated by laryngeal stridor and pharyngeal obstruction. Respiration arrest occurred only twice. Following inflation with oxygen there was an immediate and transient period of apnea followed by a return to normal respiration.

The most constant cardio-circulatory changes was a tachycardia which appeared about the time of the initial stimulation to respiration and increased progressively until the bout was terminated. When the anoxemia was most acute the heart rate was usually greater than 160. Inflation with oxygen resulted in an immediate bradycardia with an early return to normal. The systolic blood pressure increased progressively. During the period of most acute anoxia electrocardiographic tracings showed various types of arrhythmia.

The neuromuscular signs in order of appearance were twitchings about the face, generalized clonic contractions, tonic-tetanic contractions and opisthotonos or torsion extensor spasms. Consciousness was lost apparently about the time the twitching appeared. Rarely there was urinary incontinence.

Cyanosis appeared relatively late and was never intense. The pupils dilated widely.

¹ Aided by a grant from the Child Neurology Research (Friedsam Foundation).

When it was desired to terminate the bout one or two inflations with pure oxygen restored the disturbed physiological processes to normal almost instantly. The changes described are not consistent with those observed when anoxemia is coincident with narcotic depression as for example during anesthesia.

Signs of progressive acute anoxia in man. F. A. D. ALEXANDER (by invitation), BASILE LIPETZ (by invitation) and H. E. HIMWICH. Departments of Anesthesia, Neurology and Psychiatry, and Physiology and Pharmacology, Albany Medical College, Union University, Albany, N. Y. (Motion picture demonstration.)

A colored moving picture demonstration of the signs of increasing acute anoxia produced during the nitrogen anoxemia treatment of schizophrenia will be shown.

Effects of amorphous, crystalline zinc and protamine zinc insulin in animals.

FREDERICK M. ALLEN. 1031 Fifth Ave., New York City.

The physiological study of the comparative effects of different forms of insulin covers at least five points: First, the relative duration of action, which may range from more than two days for ordinary insulin to more than eight days for protamine insulin in single doses, the method likewise affording accurate tests of other forms of insulin for which intermediate periods of action are claimed. Second, the production and duration of constitutional hyperinsulinism. Third, the effect upon appetite and weight. Fourth, the toxicology, in relation to hypoglycemia and glucose administration and also independently. Fifth, the question raised by the above data as to whether the insulin action is identical except for differences in time, or whether the different forms of insulin enter into metabolism as distinct chemical substances as judged by the differences in their effects.

Studies on the central olfactory system based on the effects of brain lesions on conditioned reflexes in dogs. WILLIAM F. ALLEN. Department of Anatomy, University of Oregon Medical School, Portland.

All tests and lesions were amply controlled and all vapors inhaled were reflexly effective only over the olfactory nerves. Conditioning may precede or follow the operations. In each animal the effects of the lesions were tested by the following procedures: *a*, establishment of a conditioned response to clove vapor, which consisted of raising a foreleg within 7 seconds to break a switch that interrupted an electric shock; *b*, ability of dog to transfer this reflex to opposite foreleg; *c*, establishment of a negative response to asafetida or the differentiation between two olfactory conditioned reflexes.

Complete or nearly complete extirpation of the parietal, occipital and temporal (exclusive of pyriform and hippocampi) lobes or sectioning the fornices or total removal or coagulation of the hippocampi except extreme ventral tips produced little or no effect on procedures *a*, *b* and *c* of the previous paragraph. Ablation of the pyriform lobes and amygdaloid nuclei inhibited or prevented procedure *c*, depending on damage to this most important olfactory distributing center. Removal of the rostral ends of the frontal lobes in front of the precruciate sulcus in 4 dogs prevented procedure *c*. Complete extirpation of the motor cortex (area 4) from one side greatly delayed procedure *a* but did not effect procedures *b*

and c. Complete removal of the motor cortex from both sides prevented procedure a, but in some dogs permitted a so-called generalized response.

None of the above mentioned lesions effected food discrimination. Once having learned that a paper package might contain meat all of the operated dogs were able to select a meat packet from 3 paper packets of like size. Also their taste responses to solutions of glucose, salt, quinine and weak acetic acid were not altered.

Comparative actions of optically isomeric phenisopropylamines. GORDON A. ALLES. Laboratories of George Piness, M.D., Los Angeles, and Pharmacological Laboratory, University of California Medical School, San Francisco.

Comparative pressor actions of phenethylamine and the d-, l- and dl-forms of phenisopropylamine (benzedrine) were studied in dogs and rabbits. All these compounds produce circulatory effects of equal intensity, but the duration of the effects of the phenisopropylamines is notably longer than of phenethylamine.

On isolated rabbit ileum, phenethylamine and the d-, l- or dl-phenisopropylamines in minimally active concentrations induce an increase in tone, and in greater concentrations commonly induce a decrease in tone. The phenisopropylamines are equally effective, and all are about twice as active as phenethylamine. Phenethylamine and the phenisopropylamines inhibit the effects of epinephrine and of acetylcholine. This inhibition is dependent upon a proper molal relationship between the concentration of these amines and of epinephrine or acetylcholine.

Chloralized rabbits are strikingly awakened by proper doses of the phenisopropylamines, the awakening effects of d-phenisopropylamine being more marked than with corresponding doses of the dl- or l-isomers.

In man, following oral administration, the pressor effects of d-, l- or dl-phenisopropylamines are closely the same. The central stimulant actions of the d-isomer are, however, greater than those of the dl- or l-isomers, the d-isomer being about twice as active as the dl-isomer, and from two to four times as active as the l-isomer.

Endocrine factors in intestinal absorption. T. L. ALTHAUSEN (by invitation), M. STOCKHOLM (by invitation) and E. M. ANDERSON. Department of Medicine and Institute of Experimental Biology, University of California. (Read by title.)

The intestinal absorption of glucose has been studied in normal male and female rats and in rats in which the normal endocrine balance has been disturbed. Mature male and female rats were fasted 24 hours and then given glucose by stomach tube in amounts exceeding their capacity for absorption. After a constant interval the unabsorbed residue was determined. Normal mature male rats absorb 26 per cent less glucose than female rats.

Two weeks after removal of the gonads of mature male and female rats, the absorption rate of glucose remained unchanged in both sexes. However, when female rats were spayed at one month of age and tested two months later, the absorption of glucose was reduced to that of normal males. Castration in a corresponding group of males did not alter their absorption.

Hypophysectomy reduced the absorption of glucose by 30 to 35 per cent in both sexes but the relative difference between the sexes was unchanged. On the other hand, thyroidectomy and adrenalectomy not only greatly reduced the absorption rate but abolished the sex difference.

From the above data it is quite evident that intestinal absorption of glucose is influenced by the hormones of the thyroid and the adrenal cortex. The interrelationship of these hormones and the basis for a sex difference in absorption is not clear.

*Behavior of the sow in relation to the sex cycle.*¹ MARGARET ALTMANN (introduced by H. S. Liddell). Department of Physiology, Cornell University Medical College, Ithaca, N. Y.

Cyclic variations in the vaginal smears of the sow were compared with the variations known to occur in other mammals. Daily activity recorded by the pedometer showed a great increase during estrus periods, the peak tending to occur at the end of estrus. Often a marked depression in activity preceded the estrus period.

The total number of movements on the platform of the Pavlov frame during the daily conditioning decreased slightly in the estrus periods and specific conditioned motor activity declined.

Openings of the food box in the rest intervals between conditioned signals were more frequent during estrus than in non-estrus periods and the latent period of the motor reaction in response to a food signal was shortened during estrus. During this period the sow's disposition toward the experimenter was more often friendly. The animal less frequently tried to bite or to oppose the experimenter.

No significant changes in heart rate during the conditioning tests were associated with the estrus period. No such consistent relation between rectal temperature and the sex cycle as reported in the human female could be discovered in the sow.

The total salivary secretion collected from the parotid fistula during the daily conditioning period showed a significant reduction during estrus.

Urinary excretion of radioactive sodium and potassium in adrenalectomized rats with and without salt. EVELYN ANDERSON and MICHAEL JOSEPH (by invitation). Institute of Experimental Biology and Department of Medicine, University of California.

Radioactive sodium and potassium have been administered to adrenalectomized rats and the rate of urinary excretion of these substances determined.

Adrenalectomized rats, unsupported by salt treatment, excreted 60 per cent of the total amount of the administered radioactive sodium in 48 hours. Normal rats excreted 43.8 per cent in a corresponding period. The total sodium excretion in 48 hours in the untreated adrenalectomized rats was three times that of normal animals. The adrenalectomized rats which had been treated with NaCl postoperatively, showed excretion figures for total sodium and for radioactive sodium, which were practically the same as those for normal animals.

The potassium excretion studies showed that the adrenalectomized rats maintained on salt excrete potassium as the normal intact animal, while those not given salt have a diminished excretion of total potassium and of administered radioactive potassium.

Two cases of experimental neurosis in dogs of known genetic constitution. O. D. ANDERSON (introduced by H. S. Liddell). Department of Anatomy, Cornell University Medical College, Ithaca, N. Y.

¹ Aided by a grant from the Josiah Macy, Jr. Foundation.

In a group of 26 dogs of known hybrid stock in which salivary and motor defensive conditioned reflexes were tested for a year, two individuals developed experimental neurosis. These dogs were selected from a large number of animals employed in the experiments of Charles R. Stockard at the Cornell Anatomy Farm. An adult female from the second generation of a cross between Saluki and Basset-hound strains showed signs of shyness when the first attempts were made to establish a differentiation of salivary conditioned reflexes between a rapid metronome as signal for food and a slow metronome without food. The positive reflex, weak from the start, almost vanished when the negative signal was introduced and food was refused, the animal standing quietly throughout the experiment. After 5 months there supervened a phase of extreme restlessness and non-coöperation with absence of the salivary conditioned reflex. It was enhanced by the no-food signal and was still observed after two years of testing.

An adult male of the same genetic constitution was also timid at the beginning of the experiments. A fast metronome signalled a weak break shock to the forelimb. A slow metronome was the no-shock signal. When the slow metronome was introduced the differentiation failed to develop and the positive conditioned reflex gradually disappeared. During this phase the dog stood with a peculiar rigidity and without movement. The limbs resisted passive flexion and could be moulded by the experimenter. When he crossed the forelimbs and extended one hind limb the dog would hold such a pose for as long as 20 minutes. After a month a phase of extreme neuro-muscular activity supervened which was still maintained at the end of a year.

The spontaneous neuro-muscular activity of various pure breeds of dog and of interbreed hybrids of the first and second generation. O. D. ANDERSON (introduced by H. S. Liddell). Cornell University, Anatomy Farm, Peekskill, N. Y. (Read by title.)

From casual observation it was evident that certain breeds were far more active than others, e.g., the German Shepherd as compared with the various hound breeds. Among the dogs employed in Charles R. Stockard's investigations the amount of activity during 24 hour intervals was studied in selected individuals by means of a pedometer attached to the neck. The distribution of activity over the 24 hour period was recorded from a 5 by 22 foot pen in which the dog was confined. The floor was supported upon springs and any slight movement was registered pneumatically on a kymograph in an adjoining building.

The pure breeds observed were German Shepherd, Saluki, British Bulldog, Pekingese, Dachshund, Cocker Spaniel and Basset hound; the cross-breeds; Bassett hound and German Shepherd F₁ and F₂, Bassett hound and Saluki F₁ and F₂, Bassett hound and British Bulldog F₁ and F₂, Saluki and Pekingese F₁ and F₂ and certain cross breeds the parent stock of which we had not studied.

High, medium and low activity groups were observed. The German Shepherd, Saluki, and Cocker Spaniel fell in the high activity group. The British Bulldog, Dachshund, and Pekingese fell in the medium and the Bassett hound in the low activity group. The cross-breeds of the first generation were in the medium group. Their activity was midway between the extremes of their parent stock. Of the cross-breeds of the

second generation, most showed activity similar to the first generation of hybrids. However, a few duplicated the behavior of one or the other of the original parent pure-breed stock so that activity was often very high or low depending upon the breeds originally crossed.

Among the high activity group movements were distributed fairly uniformly throughout the 24 hours; i.e., the animals were almost as active during the night as during the day. In the medium group most of the activity occurred during the day. In the low group scarcely any movement occurred at night.

Gastric secretion in extragastric malignancy. MAX APPEL, (by invitation) and H. NECHELES. Departments of Gastro-Intestinal Research and of Pathology, Michael Reese Hospital, Chicago, Ill.

The diminution in gastric secretion which occurs in cases of gastric malignancy has long been recognized. Considerable evidence has also been produced which suggests that a similar reduction in stomach secretion is found in patients with extragastric malignancies. This observation is of considerable significance for two main reasons. First the reduction in acid content of the stomach secretion would serve as a useful diagnostic aid in questionable cases of malignancy, and secondly, it might reveal a hitherto unknown relationship between gastric secretion and malignant growths.

In an attempt to substantiate experimentally this clinical observation that extragastric malignancy produces diminished gastric acidity, gastric secretion was investigated in a series of rabbits with extensive Brown-Pearce carcinomatosis. Using a method published recently, we increased the rate and extent of spread of the tumor by previous treatment of the animals with dibenzanthracene. Gastric juice was aspirated twice weekly and free and total acid and pepsin were determined. No alteration in gastric acidity or pepsin was observed even in animals with extensive carcinomatosis. This fact combined with the known clinical observation that a high percentage of anacidity in apparently normal people is found in the same age group in which extragastric malignancy has been observed most frequently and that gastric anacidity may be present long before the appearance of a malignant tumor, suggest that reduced acid secretion of the stomach in cases of extragastric malignancy has not been established.

The effect of sodium azide on the frequency of the embryonic fish heart. C.W.J. ARMSTRONG and KENNETH C. FISHER (introduced by Laurence Irving). Department of Biology, University of Toronto, Canada, and Marine Biological Laboratory, Woods Hole, Mass.

When intact embryos of *Fundulus* or salmon are exposed to solutions of sodium azide, the frequency of the heart gradually falls to approach constancy again at a level which is below the normal by an amount depending upon the concentration of the azide solution and its pH.

The dissociation constant of HN_3 is such that over the range of pH used (pH 6 to 8) the concentration of free acid in the sodium azide solution varies markedly, and to account for the effect of pH it seems sufficient to suppose that it is only the free acid which is effective in producing the inhibition. By keeping the total azide concentration constant and varying the pH, therefore, essentially the same data is obtained as by determining the effect of different total concentrations at fixed pH. Considered

separately in this way, each of these two sets of data can be described by an equation derived from the mass action law the constants of which are similar. In addition the constants of the equation for *Fundulus* are close (if not identical) to those observed on salmon though the maximum inhibition in the two cases is different.

Though qualitatively similar, the effects of cyanide and azide differ on at least two significant points: 1, the maximum inhibition produced is not the same with the two inhibitors; 2, by inference from the mass action equations the ratio of enzyme to inhibitor in the enzyme-inhibitor complex is not the same for the two substances. The inhibition produced by cyanide is not commonly observed to vary with pH. The difference between azide and cyanide in this respect may be due to the very different pK's of the two systems.

It is concluded that the quantitative action of azide is consistent with the view that this substance slows the frequency determining process of the heart by inactivating an essential catalyst; that the effect of azide depends actually on the concentration of free HN_3 present; and that the modes of action of azide are different in essential details.

Time relations in the cyclic release of ovarian hormones in the rat. E. B. ASTWOOD (introduced by F. L. Hisaw). Biological Laboratories, Harvard University, Cambridge, Mass.

Determinations on the weight and water content of the uteri of adult rats show that the uteri reach a maximum size at proestrus and then regress to a minimum on the first day of diestrus. Their water content rises to a maximum before proestrus and then falls abruptly with the first appearance of cornified cells in the vaginal smear. Uteri of pseudopregnant animals follow the same course for the first three days, show an increase in weight and water content on the fifth day and then regress below the diestrus level. The uteri of animals in various stages of the cycle, given a single injection of 2.0 gamma estradiol six hours before death responded by a marked increase in tissue water during diestrus and preestrus. During proestrus and estrus this reaction was strongly inhibited. Evidence is presented showing that this inhibition is due to an ovarian secretion having the properties of a corpus luteum hormone. The events are taken to mean that estrogen acts unopposed during a brief period before proestrus and largely ceases to be produced prior to vaginal cornification. Corpus luteum hormone acts upon the uterus during proestrus and estrus; it is released from ovarian follicles during their preovulatory swelling and ceases to be produced at or shortly after ovulation.

Splenic rhythm in relation to blood flow. E. J. BALDES, J. H. GRINDLAY (by invitation) and J. F. HERRICK. The Mayo Foundation and the Mayo Clinic, Rochester, Minn.

"Splenic rhythm" is the expression used to designate the rhythmic changes in volume revealed by plethysmograms of the spleen. Similar rhythmic variations have been observed in continuous recordings of the flow of blood in the splenic artery and vein by Mertens.

We have studied the blood flow of the spleen in the trained dog by the thermostromuhr. Continuous photographic recordings of splenic flow were made and the observations of Mertens confirmed. The average period of these cyclic changes was forty-five seconds. During sleep and

especially after a noise these changes increased in amplitude and decreased in period. The same alterations in the cyclic changes occurred in refilling of the spleen after contraction. During contraction and while the spleen was contracted there was no rhythm. In the anesthetized dog records of blood flow in the splenic artery and vein were compared with simultaneous plethysmograms of the spleen. These showed that the variation in arterial flow was synchronous and in phase with the change in volume of the spleen; and that the change in venous flow was in phase but lagged slightly. Further observations on the denervated spleen indicated no disturbance in blood flow or volume manifestations of the splenic rhythm. Variations in blood pressure synchronous with the splenic rhythm were present when the spleen began to refill.

On the basis of this work the splenic rhythm of the dog appears to be caused, not by rhythmic contractions of the splenic musculature, but by rhythmic changes in blood flow in the splenic circulation.

Relation of anterior and posterior hypothalamic nuclei to anhydremic responses to cold in monkeys. HENRY G. BARBOUR. Department of Pharmacology and Toxicology, Yale University, New Haven, Conn.¹

Macacus Rhesus exposed to 10°-25°F for 30-40 minutes exhibits a serum concentration (specific gravity) increase of 3.5 ± 1.81 per cent. There is a parallel vapor pressure decrease of 3.3 ± 1.45 per cent. Such exposure to cold reduces the body temperature from $38.55 \pm 0.35^\circ\text{C}$ to $35.71 \pm 0.894^\circ\text{C}$. All the above means and their standard deviations are from series of 87 or more determinations.

Lesions (mostly transverse cuts) were made in definite locations involving either the anterior or posterior hypothalamus or related pathways. Depending on the position of the lesion, three groups of positive results were obtained; 1, disturbed regulation against cold (shown by excessive falls in body temperature); water shifting response undisturbed; 2, reversal of water shifting responses; regulation against heat but not cold disturbed; 3, regulation against cold disturbed and water shifting responses reversed.

Group 1 included bilateral lesions as follows: mammillary bodies (2 monkeys); mammillary bodies and lower half thalamus (3 monkeys); between preoptic nucleus and optic chiasm to center of hypothalamus (1 monkey); nerve III exit to near aqueduct (2 monkeys); hypophysectomy (3 monkeys).

Group 2 included the following lesions: both preoptic nuclei (1 monkey); preoptic nucleus, right side only (1 monkey); above preoptic nuclei to massa intermedia of both thalami (1 monkey).

Group 3 included 3 monkeys with bilateral lesions as follows: brain stem complete transection at nerve III level (2 monkeys); superior colliculi to Foramen of Monroe (low) (1 monkey).

The above described responses varied from the normal mean by two standard deviations in practically all cases.

In a further control group of eight monkeys lesions in various outlying regions were without effects upon either the responses (water or temperature) to cold. Bilateral lesions were located as follows: surface of tuber (1 monkey); surface, base of frontal lobes (1 monkey); superior colliculi

¹ These investigations were made possible by grants from the National Research Council and the Fluid Research Fund of the Yale University School of Medicine.

to aqueduct ceiling (2 monkeys); superior colliculi to aqueduct floor (1 monkey); superior colliculi through tegmentum of pons (except borders) (1 monkey); brain stem: lower half at nerve III exit (1 monkey).

It is concluded that in the monkey the water shifting responses to cold are mediated through the anterior hypothalamic nuclei. The anterior and posterior hypothalami have been confirmed respectively as centers for the regulation against heat and cold.

Analysis of iodoacetic acid effect on metabolism of isolated mammalian tissues.

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Until recently, most of the experimental data favored the hypothesis that iodoacetic acid (IAA) inhibited the oxidation of glucose in isolated tissues (cf. reviews during 1937 by Burk and by Dixon). This was in conformity with the concept of a common initial pathway for aerobic and anaerobic metabolism of carbohydrate through lactic acid. However, work by Saslow (1937) and by Stannard (1938) with frog muscle, and by the authors (Science **87**: 168, 1938), using brain cortex and skeletal, smooth, and cardiac muscle from cats and dogs yielded respiratory quotients indicative of normal carbohydrate oxidation in the presence of sufficient IAA to inhibit glycolysis 90-100 per cent. The mammalian tissues, poisoned by IAA, also oxidized added glucose.

In support of the high respiratory quotients under these conditions denoting carbohydrate oxidation, it was ascertained that they were not due to: 1, oxidation of preformed lactic acid, 2, ammonia formation from protein, or 3, poor aerobic penetration of the IAA. Further corroboration was obtained by finding quantitative disappearance of glucose in agreement with calculations based on the respiratory data.

A separation of anaerobic glycolysis and respiration was also obtained in the case of brain cortex by a procedure involving merely prolonged washing in Ringer solution without the use of poisons. The addition of small amounts of glucose showed that qualitative as well as quantitative separation could be obtained, since oxygen consumption levelled off at a glucose concentration of about 20 mgm. per cent while anaerobic glycolysis was still increasing at 200 mgm. per cent. In glucose concentrations below 25 mgm. per cent, anaerobic production of lactic acid was quantitatively unable to account for the amounts of oxygen used for the oxidation of carbohydrate.

This evidence is considered counter to any theory of carbohydrate oxidation which assumes formation of lactic acid as a necessary intermediary.

Observations on the adreno-genital syndrome. BRODA O. BARNES. Department of Medicine, University of Illinois, Chicago.

It was previously reported that the bitterling ovipositor lengthened when the fish were placed in a bath containing adequate amounts of urine or urine extracts. Tissue extracts had indicated that the active material originated in the adrenal glands and crystalline compounds from the adrenal were found to be highly potent in causing the reaction. An attempt was made to separate the material which was active on the fish

¹ This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

from the androgenic activity in the urine. It was found that after extracting the urine with chloroform and purifying the extract, most of the bitterling activity would remain in a 50 per cent methyl alcohol solution when it was shaken with carbon tetrachloride while most of the androgenic activity was removed by the carbon tetrachloride. The androgens were assayed on one day old baby chicks.

A group of female patients exhibiting varying degrees of hirsutism, amenorrhea and obesity have been studied for their excretion of androgens and the active substance on the bitterling fish ovipositor. None of the cases studied thus far have shown any increase in the excretion of androgenic activity while each was found to be putting out quantities of bitterling activity from 3 to 10 times greater than normal females. One case of proven carcinoma of the adrenal cortex has been studied and the daily excretion of bitterling activity was found to be about 50 times the normal. In general the excretion of the active substance on the fish has been found to parallel clinical impressions of the degree of activity of the adrenal cortex.

A study of the effect of adrenalectomy and iodoacetic acid poisoning on the intestinal absorption of spectroscopically active fats. RICHARD H. BARNES, ELMER S. MILLER and G. O. BURR (introduced by M. B. Visscher). Departments of Physiology and Botany, University of Minnesota, Minneapolis.

The type of unsaturation present in most natural fats, including body fat, is of an unconjugated nature. That is, if the fatty acid contains 2 or more double bonds, these double bonds are separated by at least 3 carbons. Spectroscopic measurement of such fats shows them to have very low absorption coefficients. However, if these double bonds exist in a conjugated system, i.e., only 2 carbons separating 2 double bonds, the fat has a high absorption coefficient. By prolonged saponification it is possible to convert the low spectral absorbing unconjugated fatty acids to ones with conjugated double bonds and high spectral absorption. By such a procedure the linoleic acid of corn oil, which is naturally present in an unconjugated condition, has been converted to the conjugated form with an absorption coefficient approximately 100 times greater than it had originally. This prepared conjugated fat is then distinguishable from natural body fats in its spectral absorption and can be used as a tagged fat in following such transport problems as the changes which fat undergoes while passing through the intestinal mucosa during the process of absorption from the gut.

In adrenalectomized rats 4 days after operating and showing no gross symptoms of adrenal insufficiency, which were fed the above described tagged fat, there was found no decrease in the rate of absorption from the intestinal tract and no significant change from the normal controls in the amount of tagged fat present in the phospholipids of the intestinal mucosa.

Iodoacetic acid in doses definitely toxic to rats causes a greatly decreased absorption rate of fat with a corresponding lowering of tagged fat present in the neutral fat and phospholipids of the intestinal mucosa.

Degeneration of the spinocerebellar tracts in a leopard. S. E. BARRERA and F. H. PIKE. Department of Neuropathology, New York State

Psychiatric Institute and the Department of Physiology, Columbia University, New York City.

A leopard about three years old, seen through the courtesy of the New York Zoological Society, showed weakness of the hind limbs, a slow gait, never faster than a walk, some difficulty in climbing into a cage two feet or more above the ground, and slow swaying movements of the hind quarters when standing with its fore feet elevated, especially when one hind foot was raised, as when climbing into its cage. A rather slight push to one side, directed against the hind quarters, was sufficient to make the animal fall over on its opposite side, the hind quarters going down first. It would then rise slowly and continue walking. There was no swaying of the head, and little or no swaying of the shoulders. The knee jerk was present on both sides, and reaction to touch and pressure was preserved, as shown by the reaction to stroking or patting.

The condition was of slow onset, with a history of a fall on attempting to jump some months before any marked symptoms were noticed. The fall was more probably a symptom of the early stage of the condition than a causative factor, as no injury to the spinal column was found at autopsy.

The histological examination of the spinal cord shows degeneration limited almost exclusively to the spinocerebellar tracts, with no neoplastic growth or cysts.

The case is of particular interest in that it affords a contrast between the symptoms following experimental section of the spinocerebellar tracts or lesion of the cerebellum itself, with preservation of the intraspinal connection of the descending branches of the axons arising from cells in Clarke's column and the collaterals from the ascending branches, and complete degeneration of these intraspinal connections. Considered phylogenetically, the intraspinal mechanisms of the spinocerebellar system develop before the projection system to the midbrain, medulla oblongata and cerebellum becomes very large. It is our view that the persistence of muscle tonus, so called, after cerebellar lesions is due to the preservation of this phylogenetically old intraspinal part of the cerebellar mechanism.

Effects of lesions at various levels of spinocortical sensory system in the macacus rhesus. S. E. BARRERA and A. FERRARO (by invitation).

Department of Neuropathology, New York State Psychiatric Institute and Hospital, New York City. (Read by title.)

Macacus rhesus monkeys were subjected to unilateral and bilateral block removal of the post-central convolution. Such animals in the acute stage, following the operation, revealed disinclination to use the affected limbs with ataxia, some diminution of muscle tone and preservation of the deep tendon reflexes. In unilateral cases the effect was purely contralateral. In bilateral cases the effect was bilateral. Monkeys with lesions of the same sensory system at lower levels, namely, animals with complete section of the dorsal columns in the high cervical region and with lesions of the posterior column nuclei revealed findings of the same type but varying in intensity depending upon the level. Those with section of the dorsal column were most severely affected showing practically complete disuse of the upper limbs in eating, climbing and walking with relatively greater preservation of movement in the lower limbs. The animals with dorsal column nuclear lesions occupied, with regard to intensity, a position much nearer that seen in section of the dorsal columns than that seen

with removal of the post-central convolutions. The spinocerebellar component via the dorsal columns and the external cuneate nucleus, necessarily involved in high cervical dorsal column section, is considered as being an important factor in the greater severity of symptoms associated with high cervical lesions.

Monkeys with lesions of the sensory system at various levels (dorsal columns, dorsal column nuclei and post-central convolutions). S. E. BARRERA and A. FERRARO (by invitation). Department of Neuropathology, New York State Psychiatric Institute and Hospital, New York City. (Motion picture demonstration.)

Moving pictures will be presented to demonstrate the characteristic findings in monkeys with each type of lesion discussed including high cervical section of the dorsal columns, lesions of the dorsal column nuclei (Goll and Burdach) and one or both post-central convolutions.

The blood sugar level of the fasting domestic fowl. H. T. BATT (introduced by H. H. Dukes). Department of Physiology, New York State Veterinary College, Cornell University, Ithaca, N. Y.

Studies of the carbohydrate metabolism of the domestic fowl are being made in this laboratory. Examination of the literature showed that further information about the blood sugar level in this species was needed. This report deals with the results of a study concerning the normal range of the blood sugar level in the domestic fowl under various physiological conditions.

For this work 37 healthy chickens varying in age from 6 months to 4 years, and representing 3 breeds and both sexes, were used. The blood sugar estimation, by means of the micro Folin (1928) technique, was begun as soon as the blood had been removed by venous puncture from a wing vein, no anticoagulant being utilized.

866 determinations of the blood sugar level on birds fasted for 24 hours revealed a range of from 130 to 290 mgm. of sugar per 100 cc. of whole blood. However, 57.6 per cent of the determinations resulted in values between 170 and 200 mgm. of sugar per 100 cc. of blood and 75.6 per cent in values between 160 and 210 mgm. per 100 cc.

The values obtained were analyzed to determine the effect, if any, of breed, age, sex, egg production, season of year, and the time of day at which the samples were taken. Of the conditions studied age alone seems to exert a significant effect. Immature birds tend to have a higher fasting blood sugar level than adults.

Proof of fetal swallowing, gastrointestinal peristalsis and defecation in amnio.

R. F. BECKER (by invitation), E. E. BARTH (by invitation), M. D. SCHULZ (by invitation) and W. F. WINDLE. Departments of Anatomy and Radiology, Northwestern University Medical School, Chicago.

Most information concerning activities of the fetal digestive tract is based upon deduction from gut contents or upon observation under anesthesia or partial asphyxia of the fetuses. In the present radiological study, no anesthetic was required and placental circulation was unimpaired. We withdrew 0.4 to 1.0 cc. of amniotic fluid by passing a no. 27 hypodermic needle through the abdominal and uterine walls without resorting to surgery in 27 guinea pigs between the middle of pregnancy and term

(9 weeks) and replaced it with equal amounts of colloidal thorium dioxide or hydroxide. Radiograms were obtained within a few minutes and at various intervals thereafter until birth.

From about 52 days gestation to nearly term, thorium appeared in the stomach one to four hours after injection. Usually by 24 hours so much fluid had been drunk that the sac began to clear, thorium was heavily concentrated in the stomach, and light shadows outlined the lumen of the small intestine. At forty hours, the stomach was nearly empty and the material was heavily concentrated in the small, and especially the large intestine. Segmentation of intestinal contents was clearly defined. Near term, we traced the course of thorium through the entire gastrointestinal tract and out again into the amniotic sac in the meconium. In fetuses of the sixth and seventh weeks of gestation, the passage was slower. Twenty-four hours or more elapsed before a shadow appeared in the stomach. It took about forty hours for the thorium to reach the small intestine, sixty before it appeared in the large intestine, and even after 14 days meconium had not passed.

We conclude that amniotic fluid is swallowed as early as the 42nd day of gestation in the guinea pig and soon thereafter material is passed along the intestines by peristaltic activity, concentrating in the large intestine. Defecation and meconioophagy in amino occur before the end of gestation. In no instance in which colloidal thorium was injected under normal conditions, did it appear in the fetal lungs in amino.

Autonomic responses in monkey and cat. M. B. BENDER (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn., and the Laboratories of Mt. Sinai Hospital, New York City.

Since the sympathetically denervated pupil dilates with adrenalin, and the denervated facial muscles contract with acetylcholine they may be used as indicators for exogenous or endogenous adrenalin and acetylcholine. With fright, the denervated pupil dilated strongly, whereas the denervated facial muscles contracted feebly in some but not in all cats. In the frightened monkey the denervated pupil did not dilate but the denervated facial muscles contracted vigorously in every instance. The monkey's pupil dilated slightly with fright when both the oculomotor and cervical sympathetic nerves were sectioned. Parenteral injections of cocaine reversed the fright reaction in the monkey, i.e., the denervated pupil dilated while the denervated facial muscles did not contract. Six seconds after intravenous injections of large doses of acetylcholine (0.04-0.10 mgm.) into the cat there occurred a contraction of the denervated facial muscles; 15 seconds later a pronounced dilatation of the denervated pupil followed. This indicated a delayed or reactive adrenalin effect. Intravenous injections of large amounts of acetylcholine into the uneserinized or eserinated monkey (1.0-2.0 mgm.) caused the usual contraction in the denervated face, but no dilatation of the denervated pupil such as was observed in the cat. In the same monkey intravenous injections of large amounts of adrenalin, however, produced marked dilatation of the sympathetomized pupil within 6 seconds and a definite contraction in the denervated facial muscles within 60 to 90 seconds. This indicated a delayed or reactive acetylcholine effect. There was no such effect in the cat. Intravenous adrenalin in the cat produced dilatation of the denervated pupil but no contraction in the denervated face.

These observations indicate that both parts of the autonomic system are active in each species and that the sympathetic and parasympathetic systems are in equilibrium. The cat, however, is predominantly sympathetic, while the monkey is predominantly parasympathetic in its reactions.

Dietary bradycardia and sensitivity to ouabain in the dog. A. L. BENNETT (by invitation), J. C. BURKE (by invitation) and A. R. MCINTYRE. Department of Physiology and Pharmacology, University of Nebraska Medical College, Omaha.

Six dogs (3 females and 3 males) fed autoclaved (120°C. for 5 hours) Purina dog chow and cod liver oil for four to five weeks suffered a marked weight loss and a bradycardia (50-70 per min.). The sensitivity of the heart to ouabain (g-strophanthin intravenously 0.025 mgm. per kilogram) was increased during the period of bradycardia; the P-R interval frequently being doubled (0.15 to 0.24 sec.). One-tenth milligram per kilogram of atropine sulfate intravenously at first produced A.V. block then later abolished the bradycardia. Epinephrine hydrochloride intravenously in 0.030 milligram per kilogram doses produced the same effects in the experimental animals as in the controls. The loss in weight and the normal heart-rate were not restored in the three dogs given *a*, thiamin hydrochloride intravenously 1 milligram per kilogram and thiamin hydrochloride per os; *b*, autoclaved yeast 20 grams per dog on alternate days; *c*, whole dry yeast 20 grams per dog on alternate days; *d*, fresh baker's yeast 18 grams per dog per day; *e*, fresh baker's yeast 54 grams per dog per day; and *f*, 20 milligrams vitamin C (Cebione crystals) daily per os. Animals placed on a diet of uncooked chow regained their normal heart-rates in 24 hours. We tentatively conclude that the dogs suffered a bradycardia due to an increased vagus tone not associated with a thiamin hydrochloride deficiency, and that during this condition the sensitivity to ouabain is increased.

The error of estimate of the blood cell count as made with the hemocytometer.

JOSEPH BERKSON, T. B. MAGATH and MARGARET HURN (by invitation). Division of Biometry and Medical Statistics and Clinical Pathology (Section on Parasitology), The Mayo Clinic, Rochester, Minn.

Preliminary observations of duplicated counts of erythrocytes, obtained from the records of several hospital laboratories, disclosed that the recorded differences were not as large as would be expected from statistical theory, and that apparently there existed an unconscious tendency to make repeated counts agree when enumeration is made by eye count.

An evaluation of the necessary minimum error involved in making a blood cell count by the usual methods was undertaken. Enumerations were effected by photographing the chamber field to be examined and perforating each cell in the enlarged photograph with a stylus connected with an electrical counter. By appropriate successive experiments several constituent errors of the total error were separately evaluated as follows: 1, the error of the field; 2, the error of the chamber; 3, the error of the pipette. A formula was established that estimates the total error in terms of these constituent errors for different conditions of enumeration. The formula developed for erythrocyte counts, slightly modified, was found to apply also to the estimation of the number of leucocytes.

Our results gave an estimate of the minimum error larger than that

reported by other workers. A repetition of experiments by such workers, using their technical methods, corroborated our rather than their results. We concluded that the differences in results were not due to differences of technique, but that the tendency to underestimate the differences among repeated counts, initially observed in the routine counts of hospital laboratories, was also present in the work reported from research laboratories.

The elimination in the bile of orally administered bile acids. A. L. BERMAN (by invitation), E. SNAPP (by invitation) and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Naturally occurring conjugated unoxidized bile acids in a daily oral dose of 3 or 5 grams in 32 experiments on 14 dogs increased the cholic acid output in the bile by 90 and 173 per cent respectively. The additional cholic acid secreted amounted to approximately 90 per cent of the cholates administered.

Unconjugated oxidized bile acids when administered in the same dosages to 10 dogs over periods of 3 to 5 days depressed the cholic acid output in the bile by from 0 to 32 per cent. Of the ingested oxidized bile acids 16 per cent was recovered in the bile. This quantity of keto acids more than compensated for the depression of cholic acid synthesis, so that the total bile acid output, including both cholic and keto acids, was increased over the basal output. The same doses of conjugated keto acids in 14 experiments on 9 dogs decreased cholic acid synthesis by 3 per cent. Of the ingested keto acids 7 per cent was recovered in the bile. The total bile acid output was increased over the basal output. The dog, therefore, is able to substitute readily available keto acids for its own natural bile acids in the process of bile secretion to some extent.

It will be noted that 90 per cent of the ingested cholic acid can be recovered in the bile, whereas only 7 to 16 per cent of the ingested keto acids was recovered. The following experiment revealed that this was not due to destruction entirely but to delayed elimination of keto acids. After a control period of no bile acids, the animals were given keto acids for a period of 3 or 5 days: for the following days they were given only naturally occurring bile acids. In two instances keto acids were present in significant amounts in the bile as late as the fourth day after their administration. Of the ingested keto acids 16 to 40 per cent could be recovered in the bile when the recovery period was extended four and seven days respectively.

The efferent pathway of chemoreflex vasomotor reactions of carotid body origin. THEODORE BERNTHAL and HARRY E. MOTLEY (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

In many individuals (dogs) cervical sympathectomy completely obliterated the chemoreflex vasomotor reactions which occurred in the resting submaxillary gland as a result of chemical agents (NaCN and Na_2CO_3) acting at the carotid body. However, in 2 animals in a series of 11, there were residual reactions mediated, presumably, by the parasympathetic innervation. These reactions were of lesser magnitude (30 per cent or less) than those exhibited by the completely innervated gland. Their direction and time relationships closely duplicated the latter thus indicating for these animals a reciprocal functioning of the two efferent nervous

pathways for vascular control when they are under the influence of chemoreceptor activity.

Vasomotor reactions were followed by recording the isolated venous outflow from the submaxillary gland during perfusion with normally (self) aerated whole blood under constant hydraulic conditions. The chemical agents administered were limited strictly to the region of the carotid bifurcation, a fact which was confirmed routinely by section of Hering's nerve, which procedure removed all reactions to these agents.

The determination of the excretion of beta phenylisopropylamine (benzedrine) by man. KARL H. BEYER and J. T. SKINNER (introduced by W. J. Meek). Departments of Physiology, University of Wisconsin, Madison, and Chemistry, Western Kentucky State College, Bowling Green.

From our studies, the prolonged effect of benzedrine sulfate on the metabolism and blood pressure of man suggested that the detoxication of the compound, if such occurred in the body, progressed so slowly that it might be excreted in the urine.

To test such an hypothesis it was necessary to develop a method for the detection of the amine. The principle of the reaction is the coupling of Beta phenylisopropylamine with Para nitrobenzenediazonium chloride. The NaOH or KOH solution of the resulting compound is extracted with n-butanol. Spectrophotometric analysis of the butanolic extract shows a characteristic absorption curve in the visible range, the color of the extract being predominately red. Interfering substances are eliminated by an absorption-extraction technic by means of which 1 microgram of the amine per cc. of urine can easily be detected.

Present data indicate that excretion of the drug begins within three hours after oral administration of 20 or 30 mgm. of the sulfate. About 30 per cent of the base is eliminated during the first 24 hours. Forty per cent of the amine is excreted over a period of 48 hours. Determinable amounts appear in the urine beyond 48 hours after the drug is taken.

Oxygen consumption and blood flow in perfused organs. RICHARD J. BING (introduced by Magnus I. Gregersen). Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

The submaxillary gland and the kidney of the cat were perfused with a modified Lindbergh apparatus, designed to permit anaerobic withdrawal of "arterial" and "venous" blood as well as accurate measurements of the rate of "venous outflow." Since sedimentation of the blood cells is a disturbing factor in perfusion with whole blood, it was necessary to use blood diluted with Ringer's solution (1:2) in most of the experiments.

The oxygen consumption and blood flow were measured before (in situ) and after transplantation of the organ to the pump. In each case the perfusion was carried out at a pressure corresponding to that found in the animal.

In 10 experiments on the submaxillary gland the average oxygen consumption in situ was 0.014 cc/gram/minute, and in the pump 0.003. The rate of venous outflow also fell to one-fifth of its normal value after transplantation; in perfusion experiments using whole blood the venous outflow fell to one-tenth of its normal value. In 8 experiments on the kidney the oxygen consumption dropped from 0.08 to 0.003 cc/gram/

minute and the rate of venous outflow from 1.0 cc. to 0.25 cc/gram/minute.

The cause of the decreased venous outflow and oxygen consumption was investigated on the submaxillary gland *in situ*. Interruption of the arterial blood supply to the organ for 10 to 15 minutes caused only a slight decrease in the oxygen consumption. Extirpation of the gland and retransplantation into the animal after 15 to 20 minutes reduced the oxygen consumption as much as transplantation of the organ from animal to pump. Manipulation connected with the transplantation rather than temporary oxygen lack seems responsible for the changes observed.

Salivary glands perfused with blood diluted with Ringer's solution increased in weight, whereas those perfused with whole blood showed no change in weight. Since the rate of venous outflow was decreased in both cases, one may presume that the decrease in the rate of venous outflow was not exclusively caused by edema.

B and C nerve fibers. G. H. BISHOP and JAMES L. O'LEARY (by invitation). Washington University School of Medicine, Saint Louis, Mo.

The rabbit cervical sympathetic nerve contains only *B* and *C* fibers; the nerves postganglionic to the coeliac ganglion, often only *C* fibers; the saphenous, *A* and *C* fibers but no *B*; the frog splanchnic, *A* (delta), *B*, and *C* fibers. We have investigated the thresholds of the fiber groups in these nerves to galvanic stimuli of different durations.

In general, the longer the duration of the stimulus, the less the difference between *B* and *C* voltage thresholds. At indefinite duration, in rabbit nerves, the ratio of thresholds for *B* and *C* is between 1:1 and 1:4, the most irritable *C* fibers usually responding at a lower threshold than the least irritable *B*. In the frog splanchnic the ratio is reversed, the *B-C* voltage threshold ratios being from 2:1 to 3:1. Thus two waves, *B* and *C*, which are confluent with respect to conduction rates and nearly confluent with respect to thresholds with short stimuli, are well differentiated by long-lasting stimuli, and in the frog their order of appearance with increasing strength is completely reversed. The *C* voltage threshold of frog nerves may be as low as the *A*.

This difference in properties of *B* and *C* fibers may be correlated with the polarizing effects of the current. The *B* fiber irritability curve during a galvanic current flow shows a distinct maximum at 3 to 5 m.s. (accommodation), the *C* curve shows almost none. Previous work has indicated that the *B* fibers are myelinated, the *C* unmyelinated.

An attempt is being made to count *B* and *C* fibers into size groups in nerves frozen and dried by Dr. Gordon Scott and stained on the slide with various silver techniques.

Oxygen transport by the blood of certain freshwater fish. EDGAR C. BLACK (by invitation), LAURENCE IRVING and V. SAFFORD (by invitation). Edward Martin Biological Laboratory, Swarthmore, Pa.

The respiratory properties of whole blood of freshwater fish have been determined by the construction of oxygen-dissociation curves at 15°C. The fish (catfish, bowfin, carp and common sucker) show wide variations in the pressure of oxygen required to half-saturate the blood in the virtual absence of carbon dioxide. The pressure of oxygen required to half-saturate the blood of the catfish in the absence of CO₂ is only 1.5 mm. of

oxygen. The corresponding oxygen pressures for the sucker, carp and bowfin (at 2 mm. CO₂) are 11, 4.5 and 5 mm. respectively.

Fish vary in the sensitivity to CO₂ of the equilibrium of whole blood with oxygen. Least sensitive is the blood of the catfish, in which a 4 mm. increase in CO₂ causes only 1 mm. increase in the pressure of oxygen at 50 per cent saturation. The blood of the sucker is fifteen times as sensitive to CO₂ at half-saturation, for an increase of 1 mm. CO₂ raises the pressure of oxygen 4 mm. The pressure ratios of CO₂ to oxygen at 50 per cent saturation for the bood of the bowfin and the carp are 2:1 and 1:1 respectively.

Since the environment of fresh-water fishes varies in content both for carbon dioxide and oxygen the importance of the differences in ease of oxygenation becomes quite clear. In the absence of CO₂ the blood of catfish could be saturated in the gill at pressures of oxygen intolerable to the other three fish. In the presence of CO₂ catfish could survive at low pressures of oxygen (10 mm.) while bowfin and carp would need 50 to 100 mm. oxygen. The sensitivity of the blood of suckers to CO₂ is such that not even at 150 mm. of oxygen could they survive exposure to pressures of CO₂ which would be unimportant for catfish, bowfin and carp.

But if CO₂ interferes with oxygenation at the gills of fish, it is of decided assistance when blood saturated with oxygen at low pressures of CO₂ is transported to the tissues. The CO₂ present in the tissues provides a means of raising the pressure of oxygen for diffusion to the tissues, even though the blood be first oxygenated at very low pressures of oxygen.

The assay of parathyroid extract from the calcium serum of dogs. C. I. BLISS and C. L. ROSE (introduced by K. K. Chen). The Lilly Research Laboratories, Indianapolis, Ind. (Read by title.)

A study of Miller's data on the relation between the dosage of parathyroid extract and the rise in serum calcium of dogs indicated that the efficiency of the assay could be increased considerably if the differences in susceptibility between dogs were excluded from the estimate of potency by some form of cross-test. A laboratory test standard has been adopted, therefore, and used in two experimental designs by which the differences in individual susceptibility could be segregated and the slope of the dosage-response curve determined as an integral part of the assay. The first arrangement consisted of replicated 4 by 4 Latin squares and the second of Yates' symmetrical pairs, for which the computation is described in detail. Statistical analysis of both series showed that the determination of the initial serum calcium prior to treatment could be eliminated without any loss in precision, thus reducing the usual number of bleedings and of chemical analyses by one-half. With four tests on each of 20 dogs, the unknown showed a potency equal to 130 ± 15 per cent of the laboratory standard; with two tests on each of 36 dogs, the potency of another unknown was 114 ± 13 per cent of the standard. The dosage-response curve was re-determined with the second design and confirmed the rectilinear relation between response and log-dose observed in Miller's data.

Photodynamic hemolysis. HAROLD F. BLUM, JOHN L. MORGAN (by invitation) and CHESTER HYMAN (by invitation). Washington Biophysical Institute, The Biological Laboratory, Cold Spring Harbor, and The Division of Physiology, University of California.

Percentage-time curves for hemolysis by rose bengal and light have been

measured by a photoelectrical method. These show; 1 an initial period before lysis begins, during which the light must act; and 2 a period of lysis, which may go on in the absence of light, but only if enough light has been supplied. In continuous light, 2 is dominated by light to such an extent that points on the hemolysis curve up to fifty per cent hemolysis, may be taken as a measure of the photoreaction.

The total amount of light required during the initial period is the same whether applied continuously or intermittently. The photoreaction is, thus, irreversible and additive.

The reciprocity law is obeyed approximately.

Photodynamic effectiveness is not directly proportional to the concentration of dye taken up by the cells, but theoretical calculation shows that it is dependent upon this fraction of the dye, rather than that in solution. This is borne out by comparison of the photodynamic effectiveness of eosin and rose bengal. These dyes show equal effectiveness when approximately equal concentration of dye is taken up by the cells, although to obtain this condition, the total concentration of eosin in the suspension must be about two orders of magnitude greater than in the case of rose bengal.

Photodynamic action is accomplished by oxidation of cell components by O_2 . The dye molecule acts as the light absorber, in a cyclical process in which it transfers its energy of activation most probably to a substrate molecule, returning in this act to its original state. This explains the fact that only a few per cent of the cell surface need be covered by dye in order to produce photodynamic hemolysis, since a single dye molecule acts repeatedly in this cycle, oxidizing many times its equivalent of substrate by successive capture of light quanta.

Loss of hypersensitivity to insulin in hypophysectomized dogs. R. C. DE BODO, J. E. SWEET, A. E. BENAGLIA (by invitation) and H. I. BLOCH (by invitation). Department of Pharmacology, New York University College of Medicine and Department of Surgical Research, Cornell University Medical College, New York City.

Hypersensitivity to insulin is considered one of the main characteristics of hypophysectomized animals. We have at present eight hypophysectomized dogs alive, which show an almost normal reaction to insulin. Three of these were operated upon 16 months ago, two 12 months ago and three 9 months ago. The hypophyses were removed with the technique devised by one of us (J. E. S.). All the dogs manifested a temporary diabetes insipidus. Their body weight has increased 20-70 per cent since the operation.

Insulin was given intravenously in three different doses:

(1) 0.025 unit per kilogram produced the same effect as in normal dogs, i.e., either no effect at all, or a slight effect of short duration (recovery within 40 minutes).

(2) 0.1 unit per kilogram caused in normals a drop in blood sugar of about 40 per cent with a recovery within 90 minutes. Seven of the hypophysectomized animals reacted similarly, one showed the same degree of hypoglycemia, but recovery occurred only within 180 minutes.

(3) 0.25 unit per kilogram caused in normals a drop in blood sugar of about 50 per cent, with a recovery within 120 minutes. Four of the hypophysectomized animals reacted exactly in the same manner as the normals.

The other four showed a drop in blood sugar of about 60 per cent; two showed convulsive seizures so that the experiment had to be terminated and the other two showed a delayed recovery (within 240 minutes).

In confirmation of previous workers recently hypophysectomized animals showed hypersensitivity to insulin; 0.025 unit per kilogram given intravenously produced a prolonged and very marked effect sometimes with convulsive seizures.

It is concluded that the hypersensitivity to insulin observed in hypophysectomized animals is not a permanent characteristic, but one that gradually decreases until it may finally disappear.

Gluconeogenesis in fasted hypophysectomized dogs. R. C. DE BODO, J. E. SWEET, A. E. BENAGLIA (by invitation) and H. I. BLOCH (by invitation). Department of Pharmacology, New York University College of Medicine and Department of Surgical Research, Cornell University Medical College, New York City. (Read by title.)

It is generally accepted that hypophysectomized animals when fasted show an abrupt fall in blood sugar, a decreased nitrogen excretion and are susceptible to hypoglycemic shock. Eight dogs—three of which were hypophysectomized 16 months ago, two 12 months ago and three 9 months ago—react to prolonged fasting like normal animals. The hypophyses were removed with the technique devised by one of us (J. E. S.). All the dogs manifested a temporary diabetes insipidus. Their body weight has increased 20 to 70 per cent since the operation.

These dogs were fasted for three periods of 8–12 days. During these periods they maintained their blood sugar at the postabsorptive level, but excreted smaller quantities of nitrogen than the normals under similar conditions.

It is concluded that the greatly diminished gluconeogenesis manifested in recently hypophysectomized animals is not permanent but gradually returns to normal.

Liberation by light of a melanophore stimulating substance in the blood of mammals. W. S. BOERNSTEIN (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

The level of melanophore stimulating substances in the blood was compared in rats exposed to light and in rats maintained in darkness. White rats were exposed for 5 minutes to intense illumination (Superflood no. 1 at 30 cm. distance); others kept in darkness for 3 hours. To assay the blood for melanophore stimulation, legs from freshly killed frogs were divided between two vessels, each containing 10 cc. of saline, and were treated with 3 cc. of blood from each rat. The tests were read after 1 hour in darkness. Blood, drawn from rats left in darkness, had little or no effect on the melanophores. The blood from rats exposed to light, in contrast, actively expanded the melanophores of the test frogs.

Effects of oxygen at high barometric pressure on some mammalian smooth muscle. DAVID F. BOHR (by invitation) and JOHN W. BEAN. Department of Physiology, University of Michigan, Ann Arbor.

A rhythmically contracting strip of rabbit duodenum submerged in continuously oxygenated Tyrode solution was subjected to oxygen at

gauge pressure of about 75 pounds. Photographic records were taken throughout the experiments. After $2\frac{1}{2}$ hours of exposure to the high oxygen pressure the tonus decreased gradually and became irregular; the amplitude of contractions decreased and became irregular and the uniformity of rhythm was lost. Continuation of the exposure further emphasized these changes and introduced an additional alteration, which was characterized by very pronounced contraction waves recurring at long intervals, suggestive of a periodic tonic spasm. Decompression to atmospheric pressure was attended by: a disappearance of these waves; a recovery of the precompression rhythmic contractions; and a return of the general tonus level to that obtaining before compression. Contraction amplitude however failed to recover completely. The persistence of an adverse influence of high oxygen pressure after decompression is evident in a comparison of test and control records.

An adequate explanation of these results is wanting. The lactic acid content of the bath of the preparations exposed to high oxygen was lower than that of the control preparations oxygenated at atmospheric pressure. From comparison of the effects induced by epinephrin on these preparations with those induced by oxygen at high barometric pressure it would appear that the high oxygen effects can not be attributed simply to a formation of epinephrine like substances at the neuromyal junction. The possible involvement of the myenteric plexus however has not been dismissed.

Results of experiments in which isolated rabbit iris, or the eye from which the cornea had been removed to permit rapid equilibration when exposed to high barometric pressure of oxygen in the Tyrode bath, suggest that those pupillary changes seen in the intact animal at the height of severe oxygen poisoning arise either from the C. N. S. or a circulating hormone rather than from direct effects on the nerve endings in the iris, the neuromyal junction, or the iris muscle itself.

Experimental acute yellow atrophy of the liver. W. N. BOLDYREFF and A. A. HUMPHREY (introduced by W. R. Bloor). The Sanitarium and Leila Hospital, Battle Creek, Mich.

There is in man and all the higher order of animals a periodical secretion of pancreatic and intestinal juice and bile. In dogs of average size about 30 cc. of such mixture is secreted every 2 hours. This alkaline fluid is immediately absorbed and enters the blood stream. However, a direct introduction of the same amount of this secretion kills the animal in from 1 to 2 days with manifestations of a fall in blood sugar and the number of leucocytes (Boldyreff). Introduction of the same fluid into the portal vein does not kill the animal at once, but produces an acute yellow atrophy of the liver (Humphrey and Boldyreff). Normally this periodical secretion is constantly absorbed into the blood, doing no mischief to the liver; apparently the mucuous membrane of the intestine serves as a barrier which renders harmless this destructive fluid before it enters the portal vein. Boiling causes this secretion to lose its effect. In order to determine the changes caused by the periodical fluid in the liver of animals entering there in larger amounts than normal, we introduced it into the portal vein repeatedly in sterile conditions (in dogs, cats, rabbits and chickens) at intervals of several days. Even a single introduction of this secretion caused a histological change in the liver cells, while two or three introduc-

tions produced typical acute yellow atrophy of the liver proven by microscopic investigations (Humphrey). We are now studying the effect of this fluid on the liver of animals to whom it is introduced through an intestinal fistula in considerably larger amounts than normally present (50 cc. to 100 cc. to a dog of average size). Apparently this also causes atrophy of the liver, although much more slowly. As in both man and animals the periodical activity is for unknown reasons sometimes markedly increased, for instance in pregnancy, it seems natural to assume that this is the etiological factor in the acute yellow atrophy of the liver observed clinically.

The effects of vanadium, manganese, and iron on the growth of Chilomonas paramecium. WILLIAM J. BOWEN (introduced by S. O. Mast). Department of Zoology, The Johns Hopkins University, Baltimore, Md. (Read by title.)

Vanadium augments growth in *Azotobacter* and probably in the roots of some plants. Iron and manganese are essential for the life of higher plants. Iron is essential for the life of higher animals and manganese may be. Iron is necessary for growth and maturity in some molds and augments growth in some bacteria. Manganese augments the growth in molds, yeasts, bacteria, and at least one protozoan.

The colorless biflagellate, *Chilomonas paramecium*, can be grown in culture fluid containing only simple compounds under environmental conditions accurately controlled. It therefore offers an excellent opportunity to study the effects of heavy metals on its growth.

The effects on the rate of growth in *Chilomonas* of vanadium, in VOCl_2 and Na_3VO_4 , iron, in FeCl_3 , and manganese, in MnCl_2 , in various concentrations in culture fluid were investigated by ascertaining the frequency of division in four isolated lines nearly three weeks. The concentration of VOCl_2 ranged from $1 \times 10^{-16}\text{M}$ to $1 \times 10^{-4}\text{M}$, of Na_3VO_4 from $1 \times 10^{-12}\text{M}$ to $1 \times 10^{-2}\text{M}$, of iron from $1 \times 10^{-9}\text{M}$ to $5 \times 10^{-6}\text{M}$, and of manganese from $1 \times 10^{-9}\text{M}$ to $1 \times 10^{-4}\text{M}$.

In culture fluid to which Na_3VO_4 was added no acceleration occurred but in that to which VOCl_2 was added the frequency of division increased from 3.54 ± 0.054 per day in zero concentration to 3.91 ± 0.047 in $1 \times 10^{-10}\text{M}$ and then decreased. These results indicate that completely oxidized vanadium does not accelerate growth of *Chilomonas*, but that the partially oxidized form definitely does.

In culture fluid to which no iron was added an average of 3.30 ± 0.045 divisions per day occurred. In that to which various amounts of FeCl_3 were added the frequency of division was higher, varying from 3.33 ± 0.049 divisions per day to 3.47 ± 0.045 , but there was no consistent relation between concentration of iron and frequency of division.

In culture fluid to which no manganese was added an average of 3.28 ± 0.055 divisions per day occurred. In that to which various amounts of MnCl_2 were added the frequency of division varied from 3.17 ± 0.063 to 3.40 ± 0.046 per day, but there was no consistent relation between concentration of manganese and frequency of division.

Studies on vagal inhibition of inspiration. T. E. BOYD and C. A. MAASKE (by invitation). Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago, Ill.

Dogs were used, anesthetized with barbital-sodium and with one or both

vagi sectioned. For central vagus stimulation we employed the circuit described by Schmitt and Schmitt (*Science* **76**: 328, 1932), with modifications permitting brief controllable periods of stimulation (0.1 to 1.0 sec.) at uniform frequency and shock voltage. Contacts governed by the animal's intrapleural pressure make it possible to apply such a series of stimuli regularly at any desired stage of inspiration or of expiration. Frequencies ranged from 80 to 144 per second, the voltage being just sufficient to produce, with continuous tetanization, a reflex apnea.

A considerable number of vagal volleys is required to inhibit an inspiration in progress. Once the threshold number is reached, inspiration is cut short promptly and completely; but a subthreshold series does not reduce the amplitude at all. Vagal inhibition thus affects inspiration in an all or none manner. With respect to the respiratory center, C. I. S. and C. E. S. cannot be considered to neutralize each other quantitatively.

The threshold number of stimuli required to inhibit becomes less and less as inspiration progresses, the center becoming increasingly susceptible to inhibition (cf. Boyd and Hillenbrand, *Am. J. Physiol.* **119**: 274, 1937).

With both vagi sectioned, the threshold for vagal inhibition of inspiration is not raised by increasing the respiratory dead space (up to 200 cc. for a dog of 12 kgm.). Respiration becomes more frequent, but the number of vagal volleys required at any given stage to inhibit is no greater than it is with normal dead space.

*Effects of intracoronary and intravenous injections of various drugs on the coronary blood flow.*¹ NORMAN H. BOYER (by invitation), R. WÉGRIA (by invitation) and HAROLD D. GREEN. Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O.

The effects of drugs dissolved in a minimal quantity of Locke's solution or blood and introduced directly into the coronary artery or intravenously have been studied to date during 78 injections in 16 dogs, utilizing the differential manometric method described by Green and Gregg (demonstrated at this meeting). Each determination was controlled by injection of Locke's solution alone.

Adrenalin in each case produced an increase in the rate of flow during diastole which preceded and persisted after the elevation of blood pressure. The rate of flow during systole in most instances decreased. The smallest effective intracoronary injection tried was 0.15 mgm. (30 per cent increase). One milligram increased the rate of flow as much as 100 per cent. The same dose intravenously produced 10 to 20 per cent increase of flow.

Pitressin in each case decreased both systolic and diastolic flow. The smallest effective intracoronary injection tried was 0.25 unit. One unit (intracoronary) produced almost complete cessation and intravenously approximately 30 per cent reduction of the rate of flow during diastole.

Aminophyllin and Theamin (Theophylline monoethanol amine) each increased the rate of diastolic flow, the smallest effective quantities tried being 0.9 mgm. intracoronary and 20 mgm. intravenously. Five milligrams (intracoronary) gave a maximum increase in the diastolic rate of flow of 100 per cent with very little change of blood pressure.

Sodium nitrite in some instances increased the diastolic rate of flow by

¹ Aided by a grant from the Council on Pharmacy and Chemistry of the American Medical Association.

as much as 60 per cent (30 mgm. intracoronary) or lowered the blood pressure as much as 20 mm. Hg without concomitant reduction of coronary flow (100 mgm. intravenously). The smallest effective amount tried was 20 mg. intracoronary.

Nitroglycerine increased both systolic and diastolic rates of flow. The minimum effective dose tried (0.6 mgm. intracoronary) caused 15-30 per cent increase in the diastolic rate of flow.

In most instances the blood flow returned to the control level within two minutes following the intracoronary injections and detectable changes in flow were not observable longer than 8-10 minutes after intravenous injections.

Electrophysiological studies on intestinal motility. E. BOZLER. Department of Physiology, The Ohio State University, Columbus.

The action potentials of the intestine were studied in situ and in isolated preparations by an ink-writing recorder driven by an amplifier. All types of activity of intestinal muscle are accompanied by bursts of brief action potentials not essentially different from those observed in voluntary muscle. During each pendular movement of the small intestine 1 to 3 impulses are discharged in the guinea pig, 4 to 15 impulses in the rabbit and cat, usually at a frequency of 4 to 8 per second. During the peristaltic waves there is a similar repetitive discharge lasting for a longer period. These observations can be explained on the basis of previous work on the properties of visceral muscles. It has been shown that these muscles are single physiological units like cardiac muscle, indicating that they are syncytia, and that their action potentials are due to the conduction of impulses within the muscle. The spontaneous rhythmic movements of visceral muscle are tetanic contractions. The discharge of single impulses occurs only infrequently in this type of muscle.

Masculinization of the female rat by gonadotropic extracts. JAMES T. BRADBURY (introduced by A. E. Woodward). University of Michigan Medical School, Ann Arbor.

Suckling females were selected at six days of age and placed with a mother rat in a group of eight to be raised as a litter. Sixty-four female rats have been treated with pregnancy urine extracts daily from the sixth to the thirtieth day of age. Eight litters were given two rat units of Antuitrin-S daily or its equivalent of Antophysin or A. P. L.

A definite hypertrophy of the clitoris was evident after ten days of treatment. The prepuce developed so that it could be easily drawn back to expose the clitoris. At 30 days of age the preputial glands were at least twice as large as those of the controls and an abundance of waxy secretion was easily expressed. The glans clitoridis resembled a glans penis in size and appearance. The horny spicules covering the glans were as prominent as those in the male. The cartilage of the clitoris developed to penile proportions but actual ossification was not observed. This hypertrophy was confined to the glans since there was no cavernous tissue along the urethra.

Two litters were treated with pregnant mare's serum (Gonadogen), 0.5 rat unit daily, starting on the sixth day of age. After twenty days of treatment the clitoris had enlarged to such an extent that it was difficult to replace the prepuce after it had been retracted manually. Two litters

were treated with pituitary extract (Prephysin), five rat units daily, but there was no appreciable masculinizing effect. For comparison two litters were treated with testosterone acetate (Perandren) 0.3 mgm. on alternate days.

Gonadotropic extracts of human pregnancy urine or pregnant mare's serum cause masculinization of the female rat if treatment is started at six days of age and continued for ten to twenty days. The hypertrophy of the clitoris, prepuce and preputial glands is entirely comparable to that induced by a similar course of treatment with testosterone. A gonadotropic pituitary extract did not cause any appreciable masculinization.

Changes in pH and the rate of flow of saliva accompanying pH changes in arterial blood during chorda tympani stimulation and during pilocarpine stimulation. CHARLES R. BRASSFIELD and CHARLES J. HONG (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

Studies were made of changes in pH and rate of flow of saliva from the submaxillary gland of the dog in response to changes in pH of arterial blood induced by changing O_2 and CO_2 tensions and by injecting acids and bases.

Salivary secretion was produced by continuous stimulation of the chorda tympani with a thyatron stimulator and pH changes were followed continuously in both saliva and blood by means of glass electrodes. Rate of saliva flow was followed by means of a Gibbs type of drop recorder.

Lowering the O_2 tension of the blood produced an increase in both arterial blood and saliva pH with the change in saliva being less than that of the blood. No change in the rate of flow of saliva was noted.

Increasing the CO_2 tension of the blood produced approximately the same pH changes in both blood and saliva but no change in the flow of saliva.

Lactic acid and hydrochloric acid injections produced no change in either the pH or rate of flow of saliva.

The rate of flow responses of saliva to these procedures are in striking contrast to the responses obtained when saliva secretion is stimulated by pilocarpine. The pH changes in saliva also vary quantitatively in the two responses. This constitutes further evidence that there may be a difference in the mode of action of pilocarpine and chorda tympani stimulation.

However, injecting either sodium bicarbonate or sodium carbonate produced a decrease in flow and an increase in pH of the saliva as in the case of pilocarpine stimulation. Sodium cyanide injection caused an initial increase in flow followed by a decrease as frequently occurs with pilocarpine. The pH of saliva is increased in both instances.

Factors determining the frequency of chemically initiated nerve impulses.

F. BRINK (by invitation), T. SJOSTRAND (by invitation) and D. W. BRONK. Johnson Foundation, University of Pennsylvania, Philadelphia.

An increase of the calcium ion concentration of nerve raises its threshold for electrical stimulation while a decrease in the concentration lowers the threshold. As the threshold falls the nerve finally becomes unstable and discharges a sequence of impulses. We have investigated certain of the factors which control this frequency. The results are helpful in the analysis of the basic mechanisms involved in chemical excitation.

If a restricted region of a sciatic nerve fiber of a frog is soaked in isotonic NaCl to remove the calcium ions impulses are discharged at a frequency of 10-20 per second. If now the NaCl be replaced by Na-citrate the calcium ion concentration is presumably still further reduced and the frequency of discharge increases to 100-200 per second. This activity may continue for several hours and one can therefore study the influence on the frequency of other agents which have characteristic effects upon the electrical excitability of normal axons.

The addition to the citrate of low concentrations of potassium, either as K-citrate or as KCl, gives an initial increase in the frequency followed by a decrease to a level which depends upon the concentration of the potassium.

A nerve fiber discharging impulses from a region bathed in isotonic Na-citrate will conduct through the treated portion electrically initiated impulses. It is therefore possible to cause the nerve to conduct an additional train of impulses. After the end of such a superimposed tetanus the properties of the fiber are so modified that the frequency of the chemically induced impulses is greatly reduced for some seconds.

If a region of a nerve fiber with low calcium content be asphyxiated the frequency of discharge first increases from its initial level and then decreases to zero.

The procedures which increase the frequency of impulses would have reduced the electrical threshold in normal nerve and those which decrease the frequency would have increased the threshold. The processes which normally regulate the threshold seem to control the frequency of impulses in a fiber deprived of calcium.

The glycopenic function of the adrenal cortex. S. W. BRITTON. Physiological Laboratory of the University of Virginia.

The results of many series of experiments carried out in this laboratory over several years past have demonstrated specific interrelationships between the adrenal cortex and carbohydrate metabolism. They have indeed given rise to our carbohydrate theory of cortico-adrenal function.

It now appears that in the absence of the adrenal glands the organism is unable to fix or synthesize glycogen in the liver and skeletal and cardiac muscles to any noteworthy degree. Cats were treated for varying periods of time after adrenalectomy with large amounts of glucose and saline solution by mouth, and were thus kept in apparently normal condition. On sacrifice of the animals, however, not one-tenth as much glycogen was found in the liver as in similarly-treated but unoperated animals. Exhaustion levels of liver glycogen were in fact observed. Skeletal muscle and heart glycogen values were also lower than normal by 50 per cent or more. Cortico-adrenal extract treated animals, however, under the same conditions, formed large amounts of liver, muscle and heart glycogen after adrenalectomy.

Animals without the adrenal glands were furthermore unable to form glycogen from large amounts of administered glucose in the presence of insulin, in sharp contrast to unoperated animals. The inclusion of cortico-adrenal extract in the glucose treatment of adrenalectomized animals, however, brought about large deposits of glycogen in the tissues.

These and correlated results mark the adrenal cortex as a highly effective glycopenic mechanism, apparently outranking insulin. It is suggested

indeed that the latter substance may operate, in its glycogenetic expressions, through the cortico-adrenal tissues.

Experimental adrenal insufficiency in monkeys. S. W. BRITTON and E. L. COREY. Physiological Laboratory of the University of Virginia. (Motion picture demonstration.)

Removal of the adrenal glands in monkeys is invariably fatal, usually in a week or ten days. Various species of Old and New World animals have been observed. Refusal of food is followed rather rapidly by general asthenia, and in a few hours convulsive seizures supervene. A number of convulsions occur in quick succession, followed by paralysis, coma and death. Convulsions are very similar to the hypoglycemic insulin-shock type. Besides extremely low blood glucose values, the liver glycogen is practically exhausted and muscle glycogen levels are markedly reduced.

The relation of the central nervous system to insulin sensitivity. J. R. BROBECK (introduced by S. W. Ranson). Institute of Neurology, Northwestern University Medical School, Chicago, Ill.

The existence of a hypothalamic center for control of carbohydrate metabolism has been suggested by reports of insulin hypersensitivity following lesions of this region. Such a nervous center might exert its influence by way of pathways descending through the brain stem to reach the spinal autonomic outflow, or by means of infundibular connections with the pituitary gland.

In the present investigation the blood sugar alterations following injection of 0.5 u. of insulin per kilogram body weight were studied in 77 cats with hypothalamic lesions, 5 cats with section of the cord at C₈, and 2 monkeys with section of the infundibular stalk. The tests were made weeks and months after operation, and were compared with tests performed on normal animals.

Four of the 77 cats with hypothalamic lesions were hypersensitive to insulin, exhibiting symptoms of insulin shock within three hours of insulin injection. Other cats with similar hypothalamic lesions gave normal reactions.

Blood sugar changes were essentially normal in the cats with cervical cord sections and in the monkeys with section of the infundibular stalk. Nervous connections between the hypothalamus and thoracic cord or between the hypothalamus and pituitary gland are therefore unnecessary for blood sugar recovery following administration of this dose of insulin. It seems unlikely that either of these pathways is able to compensate for loss of the other, and the possibility suggests itself that the hypersensitivity following some hypothalamic lesions is the result of some other as yet undemonstrated pathology, possibly in the anterior lobe of the hypophysis.

The elaboration of higher-order conditioned responses with food the constant incentive. W. J. BROGDEN (introduced by W. Horsley Gantt). Pavlovian Laboratory, Phipps Psychiatric Clinic, Johns Hopkins University School of Medicine, Baltimore, Md. (Read by title.)

Once conditioned forelimb-flexion from a charged grid was established to bell, a second order response was elaborated by combining a tactile stimulus with bell. Shock did not follow this combination and the tactile

stimulus was proved indifferent for flexion before being combined with bell. The tactile stimulus now evokes flexion, due to its association with bell, and response is maintained by giving food after each flexion. When the second order response is firmly established, light, first tested for indifference, is combined with the tactile stimulus, and flexion to light alone constitutes the third order. Food follows flexion to the light and tactile stimulus in combination and to the light alone. A fourth order response to tone is elaborated by the same procedure. Food is neither a conditioned nor an unconditioned stimulus for flexion, for at no time does food presented alone evoke response.

Higher-order responses could be elaborated in only 4 of 10 dogs. The second order was the most difficult to establish, the first next, then the fourth, whereas the third required the least number of trials. Successive extinctions reveal the first order to be the most resistant to extinction, the second next, the third next, and then the fourth. The retraining after each extinction took but a short time (1 or 2 days) and was about the same for all orders. There is no relationship between the speed of conditioning and the speed of extinction.

Because of failure of most of the animals to elaborate higher-order responses and the difficulty of elaboration with the successful animals, it is suggested that in general the transfer of a response from one stimulus to another, without there being association between the secondary stimuli and the unconditioned or original stimulus, takes place through substitution of formerly indifferent stimuli for each of the dual functions of the unconditioned stimulus (elicitory-function and incentive-function) instead of by a higher-order mechanism.

Postural changes in respiration. ELIZABETH BROGDON (by invitation) and FRANCES A. HELLEBRANDT. Department of Physiology, University of Wisconsin, Madison. (Read by title.)

Dependence of respiration on body position is well recognized. That deprivation of postural sway in standing may augment pulmonary ventilation to a marked degree was demonstrated in the analysis of 515 five minute records of oxygen consumption obtained upon 3 normal adults by the closed circuit method of indirect calorimetry. When standing relaxed, swaying freely, the subjects exhibited mean pulmonary ventilation increases of 36.1, 28.8 and 41.2 per cent over the horizontal values. When held in a standing position with movement mechanically restrained, they showed respectively increases of 92.8, 71.1 and 30.3 over the horizontal, figures definitely beyond any expected rise due to the increased metabolic cost of standing. For the two subjects who responded very differently to these two positions, the change in ventilation was largely a result of changed frequency of respiration. Their mean increases in rate were 66.6 and 106.7 per cent, standing rigidly, compared to 1.7 and 6.5 per cent respectively when allowed to sway. Respiratory irregularities were prominent in both standing positions. Deep inspirations were interspersed from 1 to 8 times during a 5 minute period, followed frequently by a brief apneic pause. When deprived of sway, they exhibited consistently a periodicity in amplitude so marked in one subject as to resemble Cheyne-Stokes respiration. Syncope when it occurred always followed one of these exaggerated phases.

Records were obtained while the subjects were suspended motionless in

a tank of water and while they were tilted to and held at progressively greater angles with the horizontal. In the water, pulmonary ventilation and respiratory rate for all subjects approximated the horizontal values. In the tilting experiments a progressive increase in total ventilation and rate was apparent, most marked in the case of the two subjects whose response to the rigid standing position had been greatly in excess of their response to standing with sway. These observations illustrate the relationship of postural dyspnea to an increasing hydrostatic handicap to the circulation. They suggest that the muscle pump, effective in postural sway, is relatively important in combatting the dyspnea.

The respiratory effects of localized faradic stimulation of the medulla oblongata.

JOHN M. BROOKHART (introduced by Robert Gesell). Department of Physiology, University of Michigan, Ann Arbor.

The cerebellum was removed to expose the entire length of the medulla of decorticated dogs. The medulla, from 6.0 mm. below the obex to 9.0 mm. above the obex, was systematically stimulated with fine needle electrodes, insulated except at the very tips and separated about 1 mm. in the cephalo-caudal axis. (The penetration points were in rows separated 3 mm. cephalo-caudally, and in columns separated 1 mm. from right to left, stimulations being made at intervals of 1-3 mm. from the dorsal to the ventral surface.) Small electrolytic lesions were placed to mark the points of stimulation and later located histologically.

The results of 632 observations on 57 dogs are reported. No nuclear group or fiber tract was exclusively unresponsive. No structure produced exclusively acceleratory response. No structure produced exclusively inhibitory responses, except the nucleus ambiguus which was unresponsive in one-sixth of the trials. The responses of the nucleus and descending root of V, the nuclei and fasciculi gracilis and cuneatus, the vestibular nuclei, the inferior olivary nucleus, the spino-cerebellar tracts, the spino-thalamic tracts, the lateral reticular nucleus, the medial longitudinal fasciculus, and the rubro-spinal tract were predominantly acceleratory. The responses of the nucleus and fasciculus solitarius, the nucleus ambiguus, and the reticular gray formation were predominantly inhibitory. The vestibular nuclei were predominantly unresponsive. The medial lemniscus, the medial reticular nucleus, the medial reticulo-spinal tract, the ventral reticulo-spinal tract, the lateral reticulo-spinal tract, the dorsal efferent nucleus of X, the nucleus hypoglossi, the facial nucleus and fibers, and the cell groups of the ventral horn, were approximately equally acceleratory, inhibitory, and unresponsive.

Of the four types of acceleratory and five types of inhibitory responses to be described, there was no indication of grouping in any particular structure or at any particular level. There was no indication of any group of cells or bundles of fibers having an exclusively inspiratory or expiratory function, which would lead to their being called an inspiratory or expiratory center.

Pressure and the dynamic constants of muscle. DUGALD E. S. BROWN.

Department of Physiology, New York University College of Medicine, New York City.

As Hill has shown, the relation between speed and load in an isotonic shortening may be written $(P + a)(v + b) = (P_0 + a)b = \text{constant}$

where P is the load and equals P_0 in an isometric contraction, v is the velocity of shortening (Cm./sec.), b is a constant defining the absolute rate of energy liberation and a is a constant having the dimension of a force (a gm. wt.) apparently acting as an internal frictional resistance.

With increasing temperature the rate of energy liberation, b , increases 2.05 times for a rise of 10°C . In contrast the value of a is only slightly changed, being increased slightly in proportion to the strength of the contraction. The nature of this constant force is a problem of some interest.

To examine the effects of hydrostatic pressure on contraction in terms of the above relation, a lever was constructed permitting simultaneous isometric and isotonic contraction within the pressure chamber. The results show that both a and b are decreased by pressure to practically the same extent. At 2000 lbs. a and b are decreased 45 per cent, whereas P_0 , the total tension, is reduced but 9 per cent. At 3500 lbs. a and b are decreased to 60 per cent, while the tension is reduced 25 per cent. There are several reasons for proposing that a , the resisting force, is determined by the sarcoplasmic gel surrounding the myofibrillae.

1. The great reduction in a accompanied by only a slight reduction in tension suggests that the tension developing mechanism is acting independently of a , the resisting force.

2. Pressure reduces a to the same extent as it increases the fluidity of the cortical gel in the *Arbacia* egg, and the plasma gel in *Amoeba proteus*, *Amoeba dubia*, and *Elodea*.

3. At atmospheric pressure the fluidity of the cortical gel of *Arbacia* is either unaffected or slightly decreased at the higher temperature, resembling a in this respect.

However, the parallel reduction in both a and b argues the effect of pressure on some region along the myofibrillae common to both.

Respiratory muscle action potentials under low oxygen and high carbon dioxide. RICHARD C. BROWN (by invitation), A. KEARNEY ATKINSON (by invitation) and ROBERT GESELL. Department of Physiology, University of Michigan, Ann Arbor.

Action potentials of the muscles of the dog were studied during eupnea and during hyperpnea induced by breathing uniformly prepared gaseous mixtures (4 per cent O_2 and 96 per cent N_2) and (10 per cent CO_2 , 40 per cent O_2 and 50 per cent N_2). The potentials were led from exposed muscles, through amplifiers, and recorded with a multiple oscillograph. The inspiratory muscles studied were the diaphragm, the external intercostal, and the interchondral portions of the internal intercostals. The expiratory muscles were the transthoracic, the triangularis sterni, the transverse abdominal, and the internal and external abdominal oblique.

During anoxic hyperpnea the inspiratory contractions were strongly augmented while the expiratory contractions often displayed only a brief augmentation before weakening under continued low O_2 . On returning to room air the inspiratory potentials weakened suddenly at times below eupneic intensities and then recovered more gradually towards normal. The expiratory contractions sometimes weakened to a point of disappearance. They subsequently built up to normal through a varying period of recovery.

During hypercapnic hyperpnea the inspiratory contractions were augmented as in anoxemia but the expiratory potentials showed a more

sustained augmentation during continued CO₂ excess than was the case for anoxemia. On returning to room air both inspiratory and expiratory contraction decreased slowly to their eupneic activities.

The relation of heat production to water metabolism during the administration of metabolic stimulating substances. JOHN M. BRUHN (introduced by A. D. Keller). Department of Physiology and Pharmacology, University of Alabama School of Medicine.

It has been shown that the water exchange of completely hypophysectomized dogs is sensitive to desiccated thyroid and to anterior pituitary extract administration. This report considers the relation of the metabolic response with that of the water exchange in such dogs to desiccated thyroid, anterior pituitary extract and synthetic thyroxine.

The heat production was determined daily by the open circuit method, using a small metal chamber for the animal and the Carpenter apparatus for gas analysis. Activity of the animal was continuously recorded. The dogs were maintained on a constant weighed diet and at a uniform environmental temperature of 26°C. Food was withheld for 18 hours before the run, but water was given ad libitum.

Preliminary observations indicate that the increased severity of diabetes insipidus associated with the administration of desiccated thyroid, synthetic thyroxine, or anterior pituitary extract is closely paralleled by an increase of the metabolic rate in a sensitive animal.

The fact that the water exchange responds to synthetic thyroxine indicates, we believe, that the increase of water exchange, whether brought about by anterior pituitary extract, desiccated thyroid, or synthetic thyroxine, is associated primarily with the metabolic raising principle of the thyroid. Dinitrophenol failed to increase the water exchange.

Too few experiments have been completed to state whether the rise in metabolism is proportional to the increase of water exchange. There was considerable variation between preparations in the quantitative response to a given dosage of substance of both water exchange and metabolism.

Leucocytosis following parenteral administration of liver extracts. H. D. BRUNER (introduced by A. J. Carlson). Department of Physiology, University of Chicago and Medical College of State of South Carolina, Charleston.

The neutrophilic leucocytosis occurring within 12 hours following the intramuscular administration of commercial liver extracts to normal humans presents itself as a possible means of assay of the anti-pernicious anemia principle of such extracts. The successful clinical use of liver extracts in symptomatic granulocytopenia and malignant neutropenia suggests that liver extract is granulocytopoietic.

Four normal males, 22 to 27 years, received each of the six test solutions below at one month intervals; inactivity was not enforced, but unusual exertion and drugs were avoided.

Quadruple white cell counts and complete Shilling differential counts on 200 white cells were made at one hour intervals for 12 hours after administration of the test solution and at two and three hour intervals on the days preceding and succeeding the test day. The following active anti-pernicious anemia preparations were administered in 3 cc. volumes intragluteally: Commercial liver extract, 3 cc. derived from 100 grams liver; Commercial liver extract, 1 cc. derived from 100 grams of liver;

40 mgm. Anahemin, $(\text{NH}_4)_2\text{SO}_4$ ppt. (West and Dakin); 20 mgm. Fraction H (Subbarow and Jacobson). These quantities were estimated to have approximately equal activity. A kidney extract prepared by the methods used for liver and known to be inactive, and normal saline with 0.5 per cent phenol were injected for control solutions. The order of injection was determined by chance selection.

Each subject responded qualitatively alike to the six solutions although the height of response varied. Comparison of the value at the height of response with values for the control days and for the saline-phenol injection showed that the kidney extract and the 3 cc. liver extract gave the highest average increase of total W. B. C., 65 to 70 per cent; the 1 cc. liver extract and the two purified fractions gave smaller responses, 30 to 50 per cent. The response to saline-phenol was within the limits of technical error in three of the four instances.

There was clear cut evidence of an increase in young granulocytic forms in response to but five of the 24 injections, and of these three appeared after the peak total rise.

The anti-pernicious anemia principle therefore is not responsible for the acute neutrophilic leucocytosis. The data do not support the view that the rise is due to active granulocytopoiesis.

The influence of gall-bladder distention on gastric hunger motility in the dog.

B. E. BRUSH (by invitation) and T. L. PATTERSON. Department of Physiology, Wayne University College of Medicine, Detroit, Mich.

The present investigation was undertaken to determine the effect of increased biliary pressure on the behavior of the gastric motility.

The dogs employed in this series of experiments were provided with gastric fistulae after the method of Carlson. The biliary fistula was made in the fundus of the gall-bladder which was attached to the tissues of the ventral body wall, slightly below the lower costal margin and about two centimeters to the right of the linea alba. The abdominal muscles compressed the fistulous portion of the pouch to such an extent that there was virtually no leakage of bile as was evidenced by examinations of the dressings and the stools. Even in dogs with dual fistulae surviving six months or more the weight remained constant and the hair slick. While gastric motility and tonus were being registered during hunger, pressure was applied within the gall-bladder by inflating a condom balloon of suitable size which had previously been inserted through the gall-bladder fistula. Seventy-three applications of intrabiliary pressure were made on 4 dogs, the pressure variations ranging from 38 to 210 mm. Hg. Minimal pressures below 50 mm. Hg were without effect, either on the motility or on the tonus of the empty stomach. It is apparent, therefore, that a slightly distended intrabiliary stomach does not serve as an adequate stimulus to appreciably affect the gastric motor mechanism. Pressures exceeding 50 mm. Hg in the gall-bladder balloon generally produced a temporary diminution in the amplitude of the gastric contractions or a complete inhibition of the gastric motility for periods of two to three minutes and this occurred in 90 per cent of the cases. Following these inhibitory effects on gastric motility there was usually a fall in gastric tone resulting in a changed type of contraction, increased or hypermotility, salivation, vomiting, as well as an inhibition of respiration.

The reflex nerve pathway concerned in these reactions was not determined.

Effect of exercise and rest on electrical polarity. E. L. BURGE (by invitation) and W. E. BURGE. Department of Physiology, University of Illinois, Urbana.

With the use of 1160 university students, 610 young men and 550 young women, it was found that when one non-polarizable electrode was placed on the scalp as near over the motor area of the brain as the receding of the hair would permit and another electrode on the wrist, the galvanometer connected with these two electrodes showed that an electric current of low amperage passed from the scalp to the wrist, or electrons from the wrist to the scalp.

Exercise such as running, football, basketball, wrestling, tap dancing, badminton, handball, etc. increased the strength of the current, while excessive exercise decreased it. The average strength of the current between the scalp and wrist for the 610 young men was significantly stronger than that for the 550 young women.

Similarly with the use of 682 gold fish it was found that when a gold fish was placed in water in a glass cylinder between two platinized platinum electrodes, an electric current passed from the electrode near the head to the electrode near the tail, thus confirming the observation of Michael Faraday made in 1839, one century ago, that the head of the fish (*gymnotus*) was positive and the tail negative.

It was also found that when the gold fish reversed its position in the tube a reversal in direction of the current was produced similar to reversing the connections of the galvanometer with a battery. When the fish was crosswise in the tube between the electrodes, the current decreased to zero. In fact, the position of the fish in the tube even when concealed from view could be told by the deflections of the galvanometer. It was also observed that the strength of the current was increased when the fish became active, like the human when exercising, and decreased when the fish became quiet.

Demonstration of electrical polarity in the fish and in the human. E. L. BURGE (introduced by W. E. Burge). Department of Physiology, University of Illinois, Urbana. (Demonstration.)

A gold fish is placed in water in a glass cylinder between two platinized platinum disc electrodes and these are connected to a galvanometer. It is demonstrated that an electric current of low amperage passes from the electrode near the head to the electrode near the tail, or electrons pass from the negative tail to the positive head of the fish. This demonstration confirms an observation made by Michael Faraday in 1839, a century ago, that the head of the fish (*gymnotus*) is positive and its tail negative.

By placing a non-polarizable electrode on the scalp of the human as near over the motor area as the receding of the hair permits, and one on the wrist it is demonstrated that the scalp of the human like the head of the fish is positive and the wrist, like the tail of the fish, is negative.

It is also demonstrated that with reversal of the position of the fish in the tube a reversal in the direction of the current is produced like reversing the connections of the galvanometer with a battery. When the fish is crosswise in the chamber, the current decreases to zero. It is demonstrated that the position of the fish in the tube even when concealed from view may be told by the deflections of the galvanometer. The strength of the current increases when the fish becomes active, like charging a battery, and decreases when the fish becomes quiet.

With two fish in the chamber, the current is increased when the heads of the fish are together, like connecting two batteries in parallel, but with the heads in opposite directions, the effect is neutralized and as a result the strength of the current is decreased and may be abolished.

Similarly in the human the current between the head and the wrist increases during moderate exercise and decreases during rest and sleep. During severe exercise, however, the strength of the current is decreased.

Further study on the electrical theory of anesthesia. W. E. BURGE. Department of Physiology, University of Illinois, Urbana.

A gold fish was placed in water in a glass cylinder between two platinized platinum disc electrodes. A galvanometer connected with these two electrodes showed that an electric current of low amperage passed from the electrode near the head of the fish to the electrode near its tail. The fish was then taken from the glass cylinder, placed in water in a beaker, and anesthetized by bubbling an ether-oxygen mixture through the water. The anesthetized fish was removed from the beaker, rinsed with distilled water, and replaced in the cylinder.

It was found that etherizing the fish caused a reversal in direction of the flow of current. Upon recovery from the ether, the flow of the current was again reversed to its original direction from the head of the fish to its tail. In light anesthesia there was produced a decrease in the strength of the current and not a reversal in its direction, as in deep anesthesia.

During sleep, the strength of the current in the human was decreased as in light anesthesia. A reversal in the direction of the current in ether anesthesia was found in the three human patients on whom observations have been made so far.

Burge et al in 1936 showed that when one non-polarizable electrode was placed on the exposed brain cortex of a semi-conscious, partially anesthetized dog, and another on an outlying part of the nervous system, such as the sciatic nerve, an electric current of low amperage passed from the positive sciatic nerve to the negative brain cortex. Deep surgical anesthesia caused a reversal in the direction of this current while light anesthesia only decreased its strength.

If anesthetics produce anesthesia by decreasing the negative potential of the brain cortex, or by rendering it electro-positive, then it would seem that if the electrons could be driven from the brain cortex by electrical means, thus rendering the cortex less electro-negative, or electro-positive, anesthesia would result. We have been able to produce with the use of the direct current what appears to be anesthesia.

The rôle of silicate in cataract production. W. E. BURGE. Department of Physiology, University of Illinois, Urbana. (Read by title.)

Burge showed in 1909 that human cataractous lenses from the United States and from India were alike in that there was a great increase above the normal in calcium salts, but unlike in that the cataractous lenses from the United States contained no detectable amount of silicate, whereas those from India contained an appreciable amount.

Analyses of 42 brands of table salt from the different parts of the United States showed very little if any silicate whereas analyses of the unrefined table salt used in certain parts of India (Analyses kindly supplied me by the Director of Geological Survey of India under date of June 10, 1937,

of salts from Jatta, Bahadur Khel, Darak; salt deposits in the Khewra, Warcha, and Kalabagh mines in the Punjab, and salts from MUTTRA, Kaithal, Sirsa) showed appreciable amounts of insoluble residues of sand. In parts of the Dera Ghazi Khan District, people consume what is called earth salt, which they manufacture for themselves from saline earth, and cataract is of such frequent occurrence in this region that doctors go there to gain experience in the removal of cataract.

These observations suggest that silica in the unrefined table salt and the saline earths used in India may be one of the causes for the prevalence of cataract in that country, another cause undoubtedly being the excessive ultraviolet in the tropical sunlight.

A comparison of the effect of exercise and rest on the threshold of the knee-jerk and electrical potential. W. E. BURGE. Department of Physiology, University of Illinois, Urbana. (Read by title.)

The knee-jerk was produced in the usual way, namely, by striking the patellar ligament with a hammer swung through an arc. The strength of the blow and hence of the stimulus was increased or decreased by adding or removing weights from a carrier attached to the hammer. The electrical potential and strength of the current passing between the scalp and wrist was determined by placing one non-polarizable electrode on the scalp as near over the motor area as the receding of the hair would permit and another on the wrist and connecting these two electrodes with a potentiometer or galvanometer.

Physical exercise caused the threshold of the knee-jerk to fall while subsequent rest caused it to rise. A reverse effect was produced on the potential, i.e. exercise caused the potential and amount of current passing between the scalp and wrist to increase and rest caused it to decrease.

During a day's physical work, the threshold of the knee-jerk fell so that at the end of the day, it was necessary to increase the weight of the hammer and hence the strength of the stimulus several hundred per cent to elicit a kick, while the potential and strength of the current passing between the scalp and wrist remained high during the day's activity. Upon retiring at night, the threshold rose rapidly with the onset of sleep while the potential fell.

If the subject at the end of the day remained awake and continued to work throughout the night instead of resting, the threshold of the knee-jerk continued to fall and the potential remained high. If the subject in the morning instead of arising remained in bed, rested and slept during the day, the threshold remained high and the potential low. If the subject remained awake and worked for two days and two nights, the threshold decreased so that it was difficult to elicit a kick with the addition to the hammer of several hundred grams of weight. The threshold of the knee-jerk of a night watchman fell during the night while he worked and rose during the day while he slept and rested.

*Experimental intersexuality: Correlation between treatment and degree of masculinization of genetic female rats.*¹ M. W. BURRILL and R. R. GREENE (introduced by A. C. Ivy). Department of Physiology, Northwestern University, Chicago, Ill.

¹ Supported in part by a grant from the Josiah Macy, Jr. Foundation.

Two hundred and fifty-six pregnant rats have been injected with testosterone, testosterone propionate, androsterone, dehydroandrosterone and androstenedione. One hundred and eighteen of these animals have carried to term. The female offspring have been masculinized to different degrees. The same modifications were obtained with all the androgens used.

The degree of masculinization is correlated with total amount of androgen administered and with stage of pregnancy at which treatment is begun. The highest degree of masculinization (presence of vasa, epididymides, seminal vesicles, prostates and penis with agenesis of the lower vagina) has been obtained when treatment with adequate dosages of androgens was begun before 16th day of pregnancy. The minimal total dosages required for this maximal effect were: 5.0 mgm. of testosterone propionate, 39.5 mgm. testosterone, 87.5 mgm. androstenedione, 100 mgm. androsterone and 180 mgm. dehydroandrosterone. No greater changes have been obtained with highest dosages which have been used to date: 55.0 mgm. testosterone propionate, 88.5 mgm. testosterone, 137.5 mgm. androstenedione and 800 mgm. androsterone.

Even with high dosages, the degree of effect is diminished when treatment is begun later than 16th day of pregnancy. In the female embryo, both Wolffian and Müllerian ducts are present on 16th day of development. When treatment is begun before 16th day Wolffian ducts are caused to persist and to develop in the male direction (epididymides, vasa and seminal vesicles). On the 17th day, the Wolffian ducts normally start to regress, the cranial portions being first to disappear. Treatment begun on the 17th day, therefore, can only affect those portions of the ducts which are still present, the caudal portions which give rise to the seminal vesicles. When treatment is begun later than 17th day, no Wolffian remnants persist. However, such late treatment may still cause masculinization of the urogenital sinus which normally does not differentiate until 20th day of development. Thus, treatment begun later than 17th day results in the development of the prostates and in agenesis of the lower vagina. Since the phallus is still relatively undifferentiated at birth, its differentiation may be influenced by androgenic stimulation introduced very late in pregnancy or even after birth.

Rhythmic fluctuations of sympathetic tone and their modification by temperature and by psychic influences. A. C. BURTON and R. M. TAYLOR (by invitation). Johnson Foundation, University of Pennsylvania, Philadelphia.

Plethysmographic studies of blood flow in the fingers show that its value is continually fluctuating between wide limits. The size of the volume pulsation of the digits with each heart beat is closely correlated with the measured flow, and hence may be used for a detailed analysis of the fluctuations.

In addition to the well known respiratory variations, changes occur with a much slower rhythm. Every thirty to sixty seconds periodic constrictions are seen which are simultaneous in all the digits of the body, and are accompanied by cardiac acceleration and by a rise of blood pressure. They therefore represent a rhythmic mass discharge of the sympathetic system. The connection with Traube-Hering waves is pointed out. Constrictions which are of psychic origin are also seen.

Statistical examination of results of experiments of eighty minutes'

duration shows that the distribution of intervals between constrictions is by no means random. Those of thirty to forty seconds duration are of commonest occurrence. As the environmental temperature is raised, longer periods become more evident, so that the average period increases. Also, the amplitude of the fluctuations is modified. This is small when the environmental temperature is low, greatest in the range of comfortable temperature and again less at higher temperatures.

Physical regulation of body temperature is therefore achieved, not by the setting of peripheral blood flow to an appropriate constant level, but by the modification of a fluctuation between high and low values. The intermittence of sympathetic discharge might be attributed to periodic changes of skin temperature which result in changes in the integrated sum of afferent temperature impulses. On the other hand, afferent temperature impulses may merely modify a rhythm which is an intrinsic property of the center. This latter view is suggested by a comparison of changes of skin temperature with those of blood flow and by the finding that the rhythm persists when the skin temperature is maintained constant in constant temperature water-baths.

Plasma protein changes following intravenous saline administration. D. BAILEY CALVIN. Laboratory of Biological Chemistry, University of Texas, Medical Branch, Galveston.

In previous work dealing with blood volume equilibration following intravenous saline administration it was found that the various blood constituents determined gave evidence that the establishment of equilibrium was not due simply to the removal of the injected saline solution. There seemed to be some increase in the amount of total circulating plasma protein following upon the plasma dilution.

In the earlier experiments, physiological saline in amounts equal to the dog's calculated blood volume were injected in from three to five minutes. Attempts to determine plasma volume by the dye method were inconclusive. In later experiments the blood volume was elevated by injections of 9.5 per cent sodium chloride (10 ml. per 100 ml. of calculated blood volume). There is a considerable elevation in plasma volume, which can be determined quite accurately by the dye method. The earlier indications that total circulating plasma protein increased in experimental hydremia have been demonstrated conclusively in these experiments.

The maximum rise was obtained from thirty to sixty minutes after injection. The increase was as great as fifteen to twenty per cent. At the end of four hours the plasma proteins were still elevated to some extent, although other blood constituents had returned approximately to the pre-injection level. Some variation in this response was observed, however, depending upon the urinary elimination of injected fluid and sodium chloride. If kidney function was sluggish, blood volume equilibration was less complete.

These results suggest, theoretically, a possible compensatory action on the part of the plasma protein forming organs of the body. With the fall in colloidal osmotic pressure of the blood, proteins enter the vascular system in an effort to re-establish equilibrium.

Recent work on the stimulation of lymph flow following saline injection would indicate that this may be one source for the protein increase, but the rate of increase of lymph flow, and the total increase for a thirty

minute period are too small to account completely for the rise in circulating plasma protein observed.

Further work is in progress in an effort to discover whether the increase is due primarily to albumin or globulin increment.

The effect of intra-arterially injected adrenalin on blood flow, oxygen utilization and carbon dioxide output of the intact hind leg of chloralose anesthetized cats. LEONARD CAMMER (by invitation) and FRED R. GRIFFITH, JR. Department of Physiology, University of Buffalo, Buffalo, N. Y. (Read by title.)

Continuing and amplifying previous work by Griffith and Hummel (Proc. Soc. Exp. Biol. Med. **27**: 1033, 1930) adrenalin was injected for 5 minutes into the left iliac artery at rates of 0.000,000,2 (6 experiments), 0.000,002 (5 experiments), 0.000,4 (10 experiments), and 0.002 (7 experiments) mgm. per kilo per minute; velocites of blood flow were such that the average resulting concentrations reaching the leg tissues at the beginning of injections were $10^{-10.6}$, $10^{-9.6}$, $10^{-7.2}$ and $10^{-6.4}$. Simultaneous arterial-venous blood samples and measurement of blood flow permitted determination of rates of oxygen utilization and carbon dioxide output by the tissues of the leg. Ten uninjected and 10 saline injected animals provided normal controls. Each experiment covered a half-hour period with four blood samplings at 10-minute intervals; injection occurring between samples 2 and 3. *Blood flow and oxygen utilization* showed identically parallel variation: neither was affected by the lowest rate of injection; both were decreased, roughly in proportion to dose, by the three highest rates of injection. Therefore, within this range, any effective dose was vaso-constrictor and diminished oxygen utilization. *Carbon dioxide output* was affected more complexly: maximal increase occurred with the lowest dose; the increase was proportionally less as dosage increased; until, with the maximal rate of injection, it decreased parallel with blood flow and oxygen utilization. From this it would appear that the variations in carbon dioxide output are probably for the most part without immediate metabolic significance, being the algebraic resultant of some non-oxidative process (glycogenolysis?) and effective blood flow. *Respiratory quotients*: 136 normal determinations lay within a range of 0.14 to 2.23, with a mean of 0.75 ± 0.02 and standard deviation of 0.32; for the 56 determinations after adrenalin, the corresponding statistics were 0.15 to 1.68, 0.79 ± 0.03 and 0.31. The experiments provide no clue as to the source of extra oxygen consumption associated with the calorogenic action of adrenalin on the organism as a whole.

Further observations on dogs made permanently diabetic by the administration of extracts of the anterior pituitary gland. JAMES CAMPBELL (by invitation), H. C. KEENAN (by invitation) and C. H. BEST. Department of Physiology, University of Toronto, Toronto, Canada.

Confirmation of Young's findings with regard to the production of permanent diabetes following injection of anterior pituitary extracts in dogs was reported from this department last year. These investigations have now been extended. In three animals in which the extract had produced what was apparently permanent diabetes of great severity, the pancreas has been completely removed. In the two which were observed for a long period after the pancreatectomy it was found that there was

little or no aggravation of the diabetic state as a result of this operation. The total insulin content of the pancreas of each of these three animals was less than 2 units and one contained no detectable amount of insulin. The insulin content of the pancreas of normal dogs of comparable weight is, on the average, approximately 80 units, i.e., from 3 to 4 units per gram. Extensive loss or degeneration of islet tissue was observed in all three cases and in the animal which had the most severe diabetes no normal islets were found when numerous histological sections of various parts of the gland were carefully examined. Three of the animals which we have observed have manifested approximately that degree of diabetes which would be expected to result from complete destruction of all the insulin-producing tissue. A fourth, however, was more resistant to insulin than any completely depancreatized dog we have ever observed. The resistance to insulin tended to progress throughout the whole period of observation which extended over several months after discontinuation of the pituitary injections. The metabolic rate of this animal was definitely higher than normal and was only slightly lowered by thyroidectomy. The administration of anterior pituitary extract to a completely depancreatized dog and to a dog previously made permanently diabetic by these extracts increased the daily insulin requirements from 26 and 20 units to values in excess of 86 and 160 units respectively. On ceasing the injections, the insulin requirements fell below the previous basal levels to 9 and 12 units for periods of 6 and 5 weeks respectively.

The influence of upper urinary tract distention on gastric hunger motility in the dog. K. N. CAMPBELL (by invitation) and T. L. PATTERSON. Department of Physiology, Wayne University College of Medicine, Detroit, Mich.

Clinical observations have demonstrated the apparent relationship between upper urinary tract pathology and gastro-intestinal symptomatology. Experimentally, alterations in gastric hunger motility were produced by increased pressure within the upper urinary tract as follows. A gastrostomy was performed after the method of Carlson and gastric hunger motility recorded by the balloon method. Later, a portion of the fundus of the urinary bladder was resected through a low-midline incision, the remainder of the fundus plus the trigone being sutured to the abdominal wall, thereby giving access to the ureters. The right ureter was catheterized, the catheter in turn being connected to a fluid pressure-regulating system from which any desired pressure could be introduced into the upper urinary tract at any desired rate. Gastric motility was simultaneously recorded. The following observations were made in 111 experiments on 5 dogs, 49 of which were preliminary.

Pressure variations produced in the upper urinary tract ranged from 10 to 90 mm. Hg. Low pressures, less than 35 mm. Hg, produced little or no alteration in gastric motility. Pressures exceeding 35 mm. Hg, and within the range of the maximal intrapelvic secretory pressure of the kidney, produced marked alterations in the gastric motility consisting of one or more of the following: 1, inhibition, either partial, marked, or in series; 2, a changed type of contraction; 3, increased or hypermotility; 4, tonal changes; 5, vomiting movements. The duration of pressure increase varied from 1.3 to 14.5 minutes and the duration of effects ranged

from 1 to 20 minutes. Seventy to eighty per cent of all effects were produced with pressures exceeding 35 mm. Hg, and in some fourteen trials the maximal secretory pressure of the right kidney ranged between 35 and 52 mm. Hg. Within this range effects produced were relatively proportionate to the pressure increase.

The reflex pathway by which these effects were produced was not determined in these experiments.

The effect of jaundiced serum upon the phosphatase activity of normal serum.

A. CANTAROW. Department of Medicine, Jefferson Medical College and Laboratory of Biochemistry, Jefferson Hospital, Philadelphia, Pa. (Read by title.)

The material employed consisted of serum from eight patients with obstructive jaundice (stone, carcinoma, stricture), nine patients with hepatocellular jaundice ("catarrhal" jaundice, arsphenamine hepatitis, portal cirrhosis) and six samples of serum from three dogs with hepatic damage induced by subcutaneous injection of carbon tetrachloride. The phosphatase activity of these serums ranged from 10.3 to 36 units (Bodansky).

Each serum was mixed with an equal volume of serum of essentially normal phosphatase activity (1.8-6.2 units). In each case the phosphatase activity of the mixture was practically the same as the average of the phosphatase activities of the two serums, within the limits of error of the analytic method. These observations are not in accord with the hypothesis that the increased serum phosphatase activity in jaundice is due to increased activation of a constant amount of the enzyme rather than to an actual increase in the quantity of phosphatase in the serum.

Ventricular rate in faradically induced auricular fibrillation, an index of A-V conductivity. S. A. CARLEN (introduced by L. N. Katz). Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

The ability of the A-V junctional tissues to transmit impulses from the auricle can be judged if this region is subjected to a constant, rapid bombardment of auricular impulses such as occurs in faradically induced and maintained auricular fibrillation. This was done on anesthetized dogs with chest open. The effect of the following procedures on A-V conductivity was *a*, separate, simultaneous and successive stimulation of peripheral vagi and of the stellate ganglia; *b*, injection of adrenergic and cholinergic drugs and their antagonists in completely denervated hearts, and *c*, asphyxia. The effect was measured by the alterations in ventricular rate as determined from average R-R intervals recorded in the electrocardiogram (lead II).

Stimulation of the cut vagus (10-25 sec.) caused an immediate depression of A-V conductivity followed by partial escape and by a transitory enhancement above normal after stimulation. Stimulation of the stellate ganglion enhanced A-V conductivity after a short latent period, and the persistence of effect was longer than after vagus stimulation. There was no apparent post-stimulatory depression.

Simultaneous or successive stimulation of the vagi and sympathetic nerves produced algebraically additive effects as far as magnitude and time relationships were concerned. The vagal effect was the dominant one; apparently because of the low vagal tone under barbital anesthesia.

Epinephrine caused a definite, protracted enhancement of A-V conductivity, not counteracted by ergotamine, 933 F or atropine in the doses used. Acetylcholine and mechohyl depressed A-V conductivity and the effect of the first was enhanced by eserine. Atropine annulled the action of these cholinergic drugs partially or completely.

Asphyxia, in the denervated heart, produced at first an enhanced conductivity of the A-V junctional tissues followed by depression. These changes were reversed on recovery from the asphyxia.

These experiments demonstrate the interaction of vagal and sympathetic influences and show the susceptibility of the A-V conducting tissue to cholinergic, adrenergic and asphyxial effects.

Studies on the permeability of the guinea-pig placenta to the foreign protein ricin. EMMETT B. CARMICHAEL and LOUIS C. POSEY (by invitation). Department of Physiological Chemistry, University of Alabama. (Read by title.)

There have been many studies reported on the permeability of the placenta to substances which are either normally present in the organism or are foreign to it. The following are a few of the substances that have been shown to traverse the placenta: amino-acids, allergens, bacterial antibodies, creatine, creatinine, insulin and peptone. The following modes of transmission appear feasible: 1, simple dialysis or diffusion; 2, certain vital cellular activities of the placental tissue which might be similar to those occurring either in the kidney or in the intestinal tract. However, in the case of proteins or protein cleavage products, there is the possibility that proteolytic ferments may aid in the transfer through the placenta. Since antibodies, which have been considered as related to serum globulin, pass through the placenta, we decided to investigate the permeability of the guinea pig placenta to the toxic protein of the castor bean, ricin.

Ricin was selected for these studies, because it is extremely toxic and thus would kill the mother if a very small amount should traverse the placenta from fetus to mother.

A saline solution of the ricin was injected into one fetus of each of 30 pregnant mothers. Saline solutions were injected into one fetus of each of 9 pregnant guinea pigs, and this group served as controls. The pregnant guinea pigs were near full term, and the injections were made into the fetuses after laparotomy.

All of the mothers in the control group survived, and their fetuses did not show any ill effects from the saline injections.

One fetus in each litter of the ricin-injected group was born dead and showed signs of ricin poisoning, or one still-born fetus was delivered too early to show characteristic signs of ricin poisoning, while the litter mates were alive at birth. Of the thirty pregnant guinea pigs, 24 lived and nine of these retained all fetuses from 4 to 7 days. Of the remaining fifteen recovering mothers, thirteen delivered within one day after the injection of the toxin. Three mothers were killed because of wound sepsis and evisceration. Three other mothers died from 12 to 24 hours after deliveries, and we believe that they accidentally received a small amount of the toxin at the time of the injection.

Even though certain protein-like substances have been found to pass

through the placenta, it seems that our experiments demonstrate that the guinea pig placenta is not permeable to the toxic protein ricin.

This may be due simply to the fact that ricin has a large molecule, or it may be due to some other colloidal property of the toxin. We do not know whether cleavage products of the ricin, due to the action of ferments, passed through the placenta, but it is possible since the toxicity of this protein has been shown to decrease as it was digested by proteolytic enzymes.

Human direct and indirect calorimetry with sugars. THORNE M. CARPENTER and EDWARD H. BENSLEY (by invitation). Nutrition Laboratory, Carnegie Institution of Washington, Boston, Mass.

The respiratory exchange was determined by the open-circuit method with a human subject after ingestion of 50 and 100 grams of glucose, fructose, or galactose. Simultaneously measurements were made of the changes in rectal temperature and, by the Benedict emission calorimeter, of the heat elimination. Alcohol checks on 21 days in 90 half-hour periods gave standard deviations from theory for heat, carbon dioxide, and oxygen of 2.5 per cent or less and for the respiratory quotient of 0.011. In 53 half-hour periods in 6 no-dose experiments the correlation coefficient of the differences between heat eliminated and indirect heat production and the changes in rectal temperature was -0.45 . The regression equation calculated from this coefficient showed that, when the heat lost in insensible loss (0.17 calorie) was disregarded, the correction in calories to be applied to the heat eliminated was $-0.166 + 16.3 t$ (change in rectal temperature). When the heat of insensible loss was taken into account, the regression equation gave a correction of $+0.0034 + 16.4 t$. With the application of this correction the standard deviation of the percentage differences between direct and indirect heat production in the no-dose experiments was 5.3 and the average difference with regard to sign, $+0.1$. In the sugar experiments the measured heat production (corrected by the second regression equation) was, on the average, lower than the calculated heat production by from 0.5 per cent (100 grams galactose) to 4.3 per cent (50 grams glucose). When the values were arranged according to the respiratory quotients, the directly measured heat productions in each range of quotients averaged lower than the calculated heat productions. The most significant difference was in the range from 0.95 to 1.00, the average percentage difference with regard to sign being -5.1 and without regard to sign ± 5.8 . Plotting of the percentage differences with reference to the respiratory quotients indicated that, in general, the measured was lower than the calculated heat production after glucose and fructose, and that with fructose the higher the respiratory quotient the greater was the difference.

Suppression of pituitary gonadotropic function by testosterone propionate.

HUBERT R. CATCHPOLE (by invitation) and JAMES B. HAMILTON. Laboratory of Physiology, Adolescence Study Unit, and Department of Anatomy, Yale University School of Medicine, New Haven, Conn.

The urinary excretion of follicle stimulating hormone was determined in a human male castrate at frequent intervals over a period of 10 months, during which time testosterone propionate was alternately given and with-

held. Assays were based on the increase in the weight of the uteri of 22 to 23 day old female mice, following a standardized course of 6 injections over 48 hours with autopsy at 72 hours. Before treatment, a daily excretion of 80-150 M.U. was found. Injection of 20 mgm. testosterone propionate per day for 31 days reduced follicle stimulating hormone excretion to 40 M.U. by the 13th day, and to 20 M.U. by the 23rd day. Following cessation of treatment, the pituitary output remained low for 3 days, presumably due to residual male hormone, then rose rather rapidly, approaching its original level by the 10th day, and recording 120 M.U. on the 25th day. This level was approximately maintained for 3 weeks, and it was not diminished during the following period of 3 weeks during which 60 mgm. per day of the testosterone propionate was given orally. A second course of 20 mgm. testosterone propionate per day then reduced the daily excretion to 16 M.U. by the 17th day, and cessation at the 27th day again permitted a rise of 80 M.U. or over within 24 days. Following a third course of injections of 20-40 mgm. daily, after 3.5 months there was no detectable follicle stimulating hormone in the urine. A close inverse relationship exists therefore between the follicle stimulating hormone output of the male pituitary and parenterally administered testosterone propionate.

Carbohydrate metabolism in adrenalectomized depancreatized dogs. WILLIAM H. CHAMBERS, J. E. SWEET, J. P. CHANDLER (by invitation) and A. L. LICHTMAN (by invitation). Departments of Physiology, Surgery and Biochemistry, Cornell University Medical College and New York Hospital, New York City.

Glucose balance and respiratory metabolism studies on 9 hypophysectomized depancreatized dogs have shown a limited ability to oxidize glucose, averaging 20 grams per day and varying between 12 and 32 grams. The administration of 50 grams of glucose produced a significant rise in respiratory quotient in about $\frac{1}{3}$ of the animals, from a basal level of approximately 0.76. Histological changes in the adrenal cortex could not be correlated with the carbohydrate metabolism or the amount of subdural glandular pituitary tissue found in some of the dogs.

Comparable studies therefore have been made in adrenalectomized depancreatized dogs maintained on NaCl and small amounts (2 cc.) of cortical extract (Upjohn). Carbohydrate metabolism was similar to that noted above. Basal quotients averaging 0.75 were raised after glucose administration in 2 out of 12 experiments. Urinary dextrose to nitrogen ratios varied from 0 to 2.1, basal blood sugar values between 100 and 300 mgm. per cent. Withholding cortical extract for 3 to 6 days or increasing the amount to 20 cc. did not significantly change the respiratory quotients before or after glucose administration. The diabetic condition of a depancreatized hypophysectomized dog was not improved by adrenalectomy.

The total fatty acid concentration in the blood was determined at intervals of several days in 3 animals up to 35 days in 1 instance. In each case from an initial level of over 400 mgm. per cent there was a gradual decrease to below 200 mgm. per cent. This is in contrast to the elevation previously noted by Lichtman after removal of the pancreas and pituitary. No change in blood fatty acids was observed for 12 hours after the injection of 2 to 20 cc. of cortical extract.

The results indicate approximately the same low level of carbohydrate

metabolism in the depancreatized dog after adrenalectomy (4 cases), hypophysectomy (22 cases) or both operations in one case.

Visual purple regeneration. AURIN M. CHASE and EMIL L. SMITH (introduced by Selig Hecht). Laboratory of Biophysics, Columbia University, New York City.

Regeneration of frog visual purple in 2 per cent digitalin solution has been studied with a photoelectric spectrophotometer, the density measurements being made in such a way as to reduce interference by other color changes in the solutions.

It was found that the maximum amount of regeneration takes place under these conditions at about pH 6.7. Temperature variation from 7° to 36°C. during the extraction of the retinas has no effect upon subsequent visual purple regeneration observed in the solutions. Several successive bleachings of single solutions, followed in each case by measurement of regeneration showed decreasing amounts, occurring in such a way as to indicate that a substrate is being used up in the process and that the inactivation of an enzyme is not the controlling factor.

A procedure was devised whereby the absorption of the regenerating material at several wavelengths in the visible region could be plotted for any instant during the regeneration process. Analysis of such absorption spectra showed that only a single reaction involving color change occurs during regeneration. The regenerating material absorbs slightly more at the shorter wavelengths than should be expected if only visual purple were being rebuilt. Either another colored substance is formed at the same rate as the visual purple or the regenerated visual purple is different from that originally extracted.

Measurement of the density change during the regeneration process gave data which could be fitted by a first order equation, indicating that if two molecules are involved in the process, as seems likely from studies of dark adaptation, one must be present in considerable excess.

The amount of visual purple regenerated in a number of extractions, measured at the optimum pH, ranged between 8 and 18 per cent of the visual purple present in the solution before illumination.

Distribution of metabolites in the body. JANE L. CHIDSEY (by invitation) and J. A. DYE. Department of Physiology, Cornell University, Ithaca, N. Y.

In attempting to study the rate of utilization of metabolites by the tissues of an animal, it is often necessary to know in what per cent of the body weight these substances are distributed. Laviets et al. (J. Clin. Invest. **15**: 261, 1936) found the distribution of non-metabolized substances to be in 20-28 per cent of the body weight of humans. Burn and Dale (J. Physiol. **59**: 164, 1924) from an experiment on one cat, claim the distribution of glucose to be in $\frac{1}{6}$ of the body weight. This is the figure used by Soskin and Levine (Am. J. Physiol. **120**: 761, 1937). Cori (Physiol. Rev. **11**: 143, 1931) calculated that in mice glucose was in 50 per cent of the body weight.

In a series of experiments designed to study the utilization of glucose and sodium acetoacetate injected into dogs, we found none of these figures satisfactory for all cases. Values obtained by calculating utilization from the amount of glucose injected often gave widely different results from those obtained by use of successive blood sugar levels in the same animal.

The results with acetoacetate were equally inconsistent. We have therefore studied each animal in order to determine the best figure for distribution of each substance. This figure was taken to be the one whose use gave similar results in the determination of utilization with either method of calculation. As a result of this study we believe that no one value will apply to all animals or to all metabolites. Certain general tendencies were noted, however. When the original body weight is considered, distribution is in a smaller per cent of the body weight if the animal has been eviscerated. Acetoacetate is distributed in more of the body than is glucose. And glucose injected alone seems to be distributed farther than if acetoacetate is also injected. Average distributions of metabolites in per cent of body weight are:

| | EVISцерATED DOGS | NON-EVISцерATED DOGS |
|-------------------------------------|------------------|----------------------|
| Acetoacetate | 41 (11 animals) | 49 (14 animals) |
| Glucose (with acetoacetate)..... | 22 (9 animals) | 26 (estimated) |
| Glucose (without acetoacetate)..... | 30 (13 animals) | 36 (estimated) |

Utilization of acetoacetate. JANE L. CHIDSEY (by invitation) and J. A. DYE. Department of Physiology, Cornell University, Ithaca, N. Y. (Read by title.)

Preliminary studies on utilization of sodium acetoacetate and total carbohydrate by normal dogs indicate that the injection of acetoacetate has very little, if any, effect on the disappearance of carbohydrate from the body. Twelve nephrectomized and twenty-four eviscerated nephrectomized animals, under either amytal or nembutal anesthesia, were used. The number of calories from carbohydrate covered the same approximate range for each group whether acetate was injected or not. The average number of carbohydrate calories for eviscerated animals with acetoacetate was 1.16 C/kgm./hr., and without acetoacetate 1.17C/kgm./hr. For non-eviscerated dogs there was an average of 0.96 carbohydrate calories/kgm./hr. if acetoacetate was injected, and 1.0 carbohydrate calories/kgm./hr. if it was not. In each animal which received acetoacetate, the calories from the amount of this substance which was utilized, plus those from carbohydrate utilization, are enough, or more than enough, to account for the total theoretical metabolism of the animal (i.e., more than 1.5 C/kgm./hr.). Since it has not been possible to check on the oxidation of fat from the animals' own stores, one cannot tell whether the utilization of exogenous acetoacetate produces extra calories or whether it has a sparing action on body fat. There were no outward signs (such as rise in body temperature or increased respiratory rate) that the former possibility occurred. In the absence of evidence to the contrary, therefore, it seems likely that sodium acetoacetate injected into an animal can spare body tissue. The possibility that injected acetoacetate might be stored has not been investigated. Further study of these problems is being made.

The action of proteolytic enzymes on anterior pituitary extract. BACON F. CHOW (by invitation), R. O. GREEP, (by invitation) and H. B. VAN DYKE. Division of Pharmacology, Squibb Institute for Medical Research, New Brunswick, N. J.

A single large batch of fresh hog pituitary glands was used as the source of extract. Great care was taken to avoid denaturation of protein. As far as possible all procedures were carried out at ice-box temperature. A two per cent aqueous solution of NaCl was used for initial extraction. Space permits only a brief description of subsequent operations. The hormones present were precipitated by saturation with $(\text{NH}_4)_2\text{SO}_4$ after removal of protein insoluble at pH 4.4. The precipitate, containing hormones, was dialyzed and substances insoluble in water and in dilute acid (pH 5.0) were discarded. $(\text{NH}_4)_2\text{SO}_4$ was again used, being added to one-third saturation; the precipitate appearing was discarded. $(\text{NH}_4)_2\text{SO}_4$ was then added to full saturation and the precipitate was used for our experiments. In all stages of extraction, the partition of nitrogen was followed quantitatively.

The proteolytic enzymes employed were crude trypsin (Merck), crystalline trypsin, crystalline chymotrypsin, crystalline pepsin, crystalline carboxypeptidase, and purified papain.¹ All the results, including those from appropriate controls, include attempted correlation with the percentage of protein digested; both protein and non-protein nitrogens were determined. All the assays were performed in rats hypophysectomized at an age of 21 days and included weights of the ovaries, uterus, adrenals, thyroid, and spleen.

| ENZYME | PER CENT PROTEIN DI- GESTED* | NUMBER OF RATS | AVERAGE WEIGHTS OF ORGANS IN MG. | | | | |
|--|---------------------------------------|-------------------|----------------------------------|--------|----------|---------|--------|
| | | | Ovaries | Uterus | Adrenals | Thyroid | Spleen |
| Control (no inj.) | | 4 | 8.84 | 13.2 | 6.72 | 4.93 | 100.8 |
| Extract control for papain and pepsin pH 4.57 | | 5 | 33.94 | 72.5 | 10.64 | 6.36 | 122.8 |
| Papain | 60 | 5 | 16.80 | 33.0 | 8.79 | 5.68 | 127.9 |
| Pepsin | 70 | 4 | 9.69 | 12.9 | 7.07 | 3.84 | 127.6 |
| Extract control for other enzymes pH 8.46 | | 5 | 49.98 | 65.2 | 11.83 | 6.32 | 125.0 |
| Trypsin (Merck) | 65 | 5 | 27.58 | 85.0 | 11.30 | 6.09 | 134.8 |
| Trypsin (cryst.) | 70 | 5 | 15.80 | 14.8 | 9.98 | 4.49 | 138.3 |
| Chymotrypsin (cryst.) | 75 | 5 | 17.97 | 15.1 | 9.28 | 4.10 | 128.6 |
| Carboxypeptid. (cryst.) | 12 | 5 | 21.80 | 48.0 | 10.26 | 6.46 | 105.4 |

* Corrected for hydrolysis in controls.

The relation of the liver and insulin to alcohol metabolism. BYRON B. CLARK, R. W. MORRISSEY, J. F. FAZEKAS, and C. STUART WELCH (introduced by H. E. Himwich). Departments of Physiology and Pharmacology and Surgery, Albany Medical College, Union University, Albany, N. Y.

Published work from this laboratory has demonstrated that insulin increases alcohol metabolism, and that excised diabetic liver does not oxidize significant amounts of alcohol, a function which is restored by insulin injection.

A study has been made of the relation of insulin and the liver to the rate of disappearance of alcohol from the blood. In depancreatized dogs and cats which have received no insulin for 72-120 hours, the rate of alcohol disappearance averaged 6.5 mgm. per cent per hour for dogs and 3.5 mgm. per cent per hour for cats (normal—18 mgm. per cent per hour).

¹ Most of the enzymes were secured through the kindness of Doctors Anson, Kunitz, Lineweaver, and Northrop.

The effect of insulin is chiefly on the liver. Eviscerectomized dogs oxidized insignificant amounts of alcohol averaging 3.7 mgm. per cent per hour which was unaffected by injections of insulin and glucose. This suggests that carbohydrate oxidation in the eviscerectomized animal bears no relation to the primary oxidation of alcohol. The disappearance of alcohol in the hepatectomized dog averaged 5.1 mgm. per cent per hour, a rate comparable to that of the depancreatized dog. Various observations have suggested that the fatty infiltration of the diabetic liver is a factor of secondary importance.

Our data indicate that the liver is the chief site of the initial oxidation of alcohol and that insulin is necessary for this function of the liver.

Fractionation studies on lipocaic. DWIGHT E. CLARK (by invitation), C. VERMEULEN (by invitation), PAUL B. DONOVAN (by invitation) and LESTER R. DRAGSTEDT. Department of Surgery, University of Chicago, Ill.

In the first report by L. R. Dragstedt, Van Prohaska and Harms (Am. J. Physiol. **117**: 175, 1936), lipocaic was secured in neutral alcohol extracts of pancreas rendered free of fat by extraction with ether. This product was found to contain an effective amount of lipocaic in from 1 to 1.5 grams of the dried material. Further fractionation of the dried fat-free alcohol extract has been made and the various fractions tested for activity by their ability to relieve the symptoms and signs of fatty liver in depancreatized dogs, confirmed by the bromsulphalein liver function test and by laparotomy with inspection of the liver and examination of a representative section for fat by chemical and histological methods.

When the fat-free alcoholic extract is dissolved in water, and ammonium sulphate or sodium chloride added to four-fifths saturation, the greater part of the lipocaic appears in the precipitate and the filtrate has been found to be relatively inert. The ammonium sulphate precipitate may then be dissolved in glacial acetic acid and a second precipitate secured by the addition of three or four volumes of ether to the glacial acetic acid solution. The precipitate may be redissolved in glacial acetic acid and again precipitated with ether. The precipitate, after neutralization, has been fed to depancreatized dogs and has been found to be effective in amounts of from 120 to 125 mgm. of dried substance per day.

Further fractions have been prepared by differential solubility in alcohol and a product soluble in 65 per cent alcohol has been secured that was effective on subcutaneous administration in a daily dose of from 55 to 85 mgm. of dried material.

The S-T interval in normal students. PAUL C. CLARK (introduced by Jane Sands Robb). Department of Pharmacology and the Student Health Service, College of Medicine, Syracuse University, N. Y.

As part of the student health program in this University, electrocardiograms are taken on medical students. One hundred and ninety students have provided 220 records. The most frequent abnormality found is positive shifting of the S-T interval. A shift of 1 mm. or more occurred in lead 1 in 38 per cent, in lead 2 in 50 per cent, in lead 3 in 54 per cent, in lead 4F in 55 per cent, and in lead 4R in 79 per cent. Although displacements of 1 mm. have no pathological significance, nevertheless 39 students (20.5 per cent) have more extreme shifts of from 2 to 5 mm.

None of these students had a history of cardiac illness, clinical signs of heart disease, acute illness when the records were taken, and none were taking drugs. Until the cause of such shifts is known, little can be said regarding prognosis. Auricular T wave complicating the S and prolongation of the QRS have each been suggested as a cause. Others have related S-T shifts to potentials caused by injured but not dead cells. Probably such are not present here though not definitely proven absent. In the dog such marked shifts of S-T are not produced by displacement or rotation of the heart, even when the pericardium is open and maximum axis deviation is produced. In no record is QSR over 0:1 second and generally it is about 0.06 second. No students had negative T waves in leads one or two. Cardiac scarring, local variation in conduction time, unusual heart position in relation to electrodes, or anomaly of the conducting system might cause such displacements. One may conclude that since up to 20 per cent of normals show S-T shift of 2 or more mm., one should be cautious in diagnosing myocardial damage in the absence of history and physical signs. Only time will tell whether these shifts are the early signs of progressive change and only accidental death with autopsy will prove whether such shifts occur in the absence of pathology. The Hendricks Research Fund supported this work.

Effect of histamine pretreatment upon some physiological changes following adrenalectomy. WILLIAM G. CLARK (by invitation) and EATON M. MacKAY. The Scripps Metabolic Clinic, La Jolla, California, and the Department of Zoology, University of Minnesota, Minneapolis.

It has been demonstrated in a previous study (MacKay and Clark, Proc. Soc. Exper. Biol. and Med. **39**: 56, 1938) that histamine pretreatment exerts a protective action upon the adrenalectomized organism in that the survival time after adrenalectomy and nephrectomy was increased and there was an increased resistance to potassium toxicity after adrenalectomy in the rat. Extending these experiments, histamine diphosphate, administered in daily subcutaneous doses starting with 50 mgm. and totaling over 500 mgm. in an 11 day period exerted a protective effect upon the usual decrease in the apparent blood volume, serum sodium and body temperature (rectal) as well as upon the increase in hemoglobin concentration in the subsequently adrenalectomized rat subjected to muscular exertion.

No effects of histamine pretreatment were observed on the performance of muscular work, the level of the liver or muscle glycogen or blood sugar, the toxic effects of hypertonic glucose solutions administered *per os* or the growth rate in young rats after subsequent adrenalectomy.

In confirmation of the reports of others adrenalectomy increased the sensitivity of the albino rat to histamine, about 100 \times . One or 2 subcutaneous injections (100 mgm.) of histamine diphosphate 12-24 hours before adrenalectomy increased the resistance of adrenalectomized rats to lethal doses of histamine administered either subcutaneously or intraperitoneally. Histamine injections, repeated over a long time or in one large dose 12 hrs. before, produced 13-31 per cent hypertrophy of the adrenal gland of the rat.

A tolerance to histamine could not be developed in the guinea pig by daily subcutaneous injections of 0.1 mgm. per 100 grams B. W. over two weeks or by increasing this dosage to 0.3 mgm. within two weeks' time. The survival time after 0.5-0.6 mgm. per 100 grams B. W. in such pre-

treated animals was not greater than that in the controls. Such pre-treatment likewise had no effect upon survival time after adrenalectomy in the guinea pig.

Changes in intestinal volume of cats in adrenal insufficiency produced by pressor drugs and splanchnic nerve stimulation. R. A. CLEGHORN, J. L. A. FOWLER (by invitation) and J. S. WENZEL (by invitation). Department of Medicine, University of Toronto, Canada.

In the terminal stage of adrenal insufficiency in cats splanchnic nerve stimulation and pressor drugs, with the exception of epinephrine, are almost without effect on the blood pressure. (Elliott, 1914; Armstrong, Cleghorn, Fowler and McVicar, 1939). In order to determine whether such procedures cause any vasoconstriction of the dilated splanchnic vessels in adrenal insufficiency and to observe the reaction of the intestinal smooth muscle, plethysmographic studies of the small intestine were made. Adrenalectomized cats showing signs of severe adrenal insufficiency were used, and starved animals acutely adrenalectomized served as controls. Blood pressure and gut volume were recorded simultaneously and direct observation of the intestines made possible by having a glass top to the plethysmograph. In severe adrenal insufficiency with the blood pressure at 40 mm. Hg and lower, splanchnic nerve stimulation may cause quite marked constriction of the splanchnic vessels though little rise in blood pressure. The gut does not relax as in controls. With epinephrine, marked vasoconstriction occurs followed by an increase in gut volume due to relaxation. Pitressin produced marked decrease in gut volume with strong contraction of the intestine. The injection of barium chloride is followed by a great increase in volume and activity of the intestine with striking visible congestion.

Electric potentials in the medial geniculate body of the cat. JOHN D. COAKLEY (by invitation) and E. A. CULLER. The University of Rochester, N. Y.

The medial geniculate body of the cat is being explored with co-axial electrodes to determine whether the various audible frequencies involve separate pathways in this nucleus and, if so, where these tracts are located.

The peculiar difficulty of the problem is, that the acoustic potentials are so extremely minute as to lie within the noise-level of the best modern amplifiers; they are often less than one-tenth as large as those found in the optic thalamus. For this reason previous investigators have resorted to complex, impulsive sounds (clicks). By equipping a high-gain amplifier with a narrow band-pass filter, continuously variable from 3 to 14,000 cycles, we can detect signals far below the unfiltered noise-level. Fourier analysis can be made directly and wave-form determined by reassembling the measured components. Results to date indicate that pulses traversing the medial geniculate are low, broad spikes synchronized with the stimulus at least as high as 2,000 cycles.

Data are not yet adequate to reveal unambiguously the site of maximal potential for specific frequencies. It is abundantly clear, however, that the magnitude of deflection depends upon the position of the electrode within the nucleus. It also appears that a particular frequency activates a considerable portion of the nucleus, but that maximal activity is confined to a restricted region.

That the potentials are due *solely* to the activity of the geniculate tracts is indicated by this evidence:

1. They are absent while the electrode is near but outside the medial geniculate, appear while it traverses the nucleus, and again vanish as it leaves through the opposite border.

2. The shielded electrode proves to be insensitive to large potentials 1 mm. distant; hence is not affected by spread from the cochlea or other acoustic centers.

3. The pulses are concomitant with acoustic stimulation and fail to appear when other forms of stimulation are applied.

4. They are absent in cats with serious middle-ear infection.

5. Administration of ether in small amounts abolishes them completely.

6. They disappear just prior to death.

*Experiments on the interaction of hypoglycemia and anoxia.*¹ ANN COFFEE (by invitation) and ERNST GELLHORN. Department of Physiology, College of Medicine, University of Illinois, Chicago.

Gellhorn, Ingraham and Moldavsky (1938) found that the blood pressure response to low oxygen increases during insulin hypoglycemia with falling blood sugar. Since this reaction occurs even after adrenalectomy it is thought to be due to increased sympathetic stimulation.

In order to further study the influence of anoxia on the sympathetico-adrenal system in relation to the blood sugar level, 105 experiments were undertaken on nonnarcotized rabbits on the influence of anoxia on the blood sugar. It was found that in control animals the inhalation of 7 per cent O₂ for 15 minutes produces a slight temporary hyperglycemia which subsides a few minutes after the readmission of air. If, however, the same period of anoxia is administered 45 minutes after the subcutaneous injection of 0.25 unit of insulin per kilogram it is found that it has a more profound effect, thereby causing a rise in blood sugar above the sugar level found in the insulin control experiment. This effect extended over a period of two hours.

The experiments indicate that anoxia is a more powerful stimulant to the sympathetico-adrenal system in hypoglycemia than at normal blood sugar levels. If, however, the period of anoxia is prolonged over a period of two hours the hypoglycemic effect of insulin is aggravated.

The results may be tentatively explained on the assumption that anoxia, when administered for a short period, stimulates the sympathetico-adrenal system in hypoglycemia more than at normal blood sugar levels. However, if it is administered over longer periods of time, it paralyzes this system and a more severe hypoglycemia results.

Anaerobic glutamic acid formation in different tissues. PHILIP P. COHEN² (introduced by H. C. Bradley). Department of Biochemistry, University of Sheffield, England. (Read by title.)

The rate of transamination ("Umaminierung," a reaction discovered by Braunstein and Kritzmann, *Enzymol.*, **2**: 129, 1937) has been determined in a number of minced and sliced surviving tissues. The course of the reaction was followed by measuring the rate of glutamic acid formation from alpha ketoglutaric acid and 1 (+) alanine under anaerobic conditions

¹ Aided by a grant from The John and Mary R. Markle Foundation.

² National Research Council Fellow in Medicine.

(40°C, pH 7.4, saline-phosphate or saline-bicarbonate, final substrate concentration 0.016M). Glutamic acid was determined by the micro-method recently described by Cohen (Chem. Indust. 58: 55, 1939).

The rates of glutamic acid formation, expressed as milligrams of glutamic acid per hour per gram dry weight of tissue, of the different tissues are as follows: pigeon breast muscle, 400; guinea pig kidney cortex, 72; pigeon gizzard, 66; rat testis, 60; rat liver, 46; guinea pig brain, 26; rat duodenum, 24.2; sheep retina, 19.2; rat lung, 19; rat amnion, 18.6; sheep kidney medulla, 16.4; sheep kidney papillae, 15.8; rat pancreas, 9; rat placenta, 4.2; guinea pig spleen, 4; guinea pig adrenal cortex, 3.6; rat (pregnant) uterus, 3.3; guinea pig adrenal medulla, 2.8; rat spleen, 2.

Pigeon breast muscle is the most active tissue. The rate of transamination is such that for every molecule of oxygen used by this tissue, one molecule of glutamic acid can be formed anaerobically from alpha ketoglutaric acid and 1 (+) alanine.

While the significance of the transamination reaction is not clear, the rapid rate suggests that it is of great metabolic importance in many tissues.

Electrical impedance of the squid giant axon during activity. KENNETH S. COLE and HOWARD J. CURTIS (by invitation). Department of Physiology, Columbia University, New York City.

Alternating current impedance measurements have been made over a wide frequency range on the giant axon from the stellar nerve of the squid, *Loligo pealii*, during the passage of a nerve impulse. The transverse impedance was measured between narrow electrodes on either side of the axon with a Wheatstone bridge having an amplifier and cathode ray oscillograph for detector. When the bridge was balanced, the resting axon gave a narrow line on the oscillograph screen as a sweep circuit moved the spot across. As an impulse passed between the impedance electrodes after the axon had been stimulated at one end, the oscillograph line first broadened into a band, indicating a bridge unbalance, and then narrowed down to balance during recovery.

From measurements made during the passage of the impulse and appropriate analysis, it was found that the membrane phase angle was unchanged, the membrane capacity decreased about two per cent from its resting value of $1 \mu f/cm^2$, while the membrane resistance fell from a resting value of 1000 ohm cm^2 to an average of 25 ohm cm^2 . The onset of the resistance change occurs somewhat after the start of the monophasic action potential but coincides quite closely with the point of inflection on the rising phase, where the membrane current reverses in direction, corresponding to a decrease in the membrane electromotive force.

The electromotive force and the conductance are closely associated properties of the membrane, and their sudden changes are themselves, or are due to, the activity which is responsible for the all-or-none law and the initiation and propagation of the nerve impulse. These results correspond to those previously found for *Nitella* and lead us to expect similar phenomena in other nerve fibers.

Membrane resistance of the squid giant axon at rest. KENNETH S. COLE and ALAN L. HODGKIN (by invitation). Department of Physiology, Columbia University, New York City. (Read by title.)

The direct current longitudinal resistance of the giant axon from the stellar nerve of the squid, *Loligo pealii*, has been measured as a function

of the electrode separation. Large sea water electrodes were used and the inter-electrode length was immersed in mineral oil. The slope of the resistance vs. length curve is large for small electrode separations but becomes smaller and finally constant as the separation is increased.

An analysis of the resistance vs. length curves has been made, following cable theory, with the following results. The axon membrane has a resistance at rest of about 1000 ohm cm². The protoplasm has a specific resistance of about 1.4 times that of sea water. The resistance of the connective tissue sheath outside the fiber corresponds to a layer of sea water about 20 μ in thickness. The characteristic length for the axon is about 2.3 mm. in oil and 6.0 mm. in sea water.

The respiratory and cardio-vascular response to repeated occlusions of the head arteries in nembutalized cats. HELEN C. COOMBS. Department of Physiology and Biochemistry, New York Medical College, Flower and Fifth Avenue Hospitals, New York City. (Read by title.)

Two groups of twenty cats each were used. One group was etherized until the initiation of the first occlusion of the head arteries. The other group was nembutalized (0.4-0.6 grain per kilo body weight about an hour before the experiment). The cerebral arteries were isolated ready for temporary occlusion with seraffins, after the manner described by Stewart and Pike in 1907. Respiration and blood pressure were recorded. In each group, ten animals were intact and ten were vagotomized after a control occlusion. Periods of complete occlusion of the cerebral vessels of about three minutes alternated with periods of resuscitation (with artificial respiration) of from ten to twenty minutes, or until adequate respiration and blood pressure had returned.

In the group of intact etherized cats, from 10 to 18 occlusions could be done before final failure of respiration and blood pressure, and from 7 to 14 occlusions could be done on vagotomized cats. This is in line with much previous work of Pike and Coombs.

Following nembutal administration the total number of occlusions obtainable was not less than two nor more than six before respiratory failure. Blood pressure remained at a level of 100 mm. Hg or higher, but the reappearance of regular respiration following reestablishment of cerebral circulation was no guarantee of its permanence. Within a short time after intermission of artificial respiration, blood pressure being above 100 mm. Hg, for no apparent reason, respiration would become gasping and fail unless artificial respiration were again employed. The form of the failing respiration was like that shown after section behind the posterior colliculi. (Coombs, H. C. Am. J. Physiol. **46**: 459, 1918.)

Division of the vagi did not cut down the number of occlusions or impair the anemic response as to height or duration. The cats were able to withstand from two to four more occlusions than the intact cats. Stimulation of the sciatic nerve in this series showed a cardio-vascular response at times when no respiratory response was elicitable. Apparently, in nembutalized cats under alternate periods of anemia and resuscitation, the respiratory center fails earlier than the cardio-vascular.

The effects of renin and pitressin on renal blood flow and clearance. A. C. CORCORAN (by invitation) and IRVINE H. PAGE. Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Ind.

Renal blood flow was calculated from the clearances and extraction

percentages of phenol red and inulin in uni-nephrectomized female dogs during control periods, intravenous infusion of a renal pressor substance "renin," of post-hypophyseal pressor substance "pitressin" before and after injection of atropine.

The renal plasma clearances of phenol red and inulin varied directly with calculated renal plasma flow during control periods. The renal extraction percentages tended to increase with decreasing renal plasma flows.

Renal plasma flow fell sharply in 9 of 10 experiments in which "renin" was infused. The fall of flow was associated with a marked increase of inulin extraction in 8 experiments, so that inulin clearances were maintained near the control levels. Phenol red clearances fell roughly in proportion to the fall of renal plasma flow. Blood pressure was increased. Skin temperature was not altered.

"Pitressin" was infused in 11 experiments, increasing renal plasma flow in five, reducing it in 4 and not affecting flow twice. Inulin extraction was sharply increased in the four experiments in which renal plasma flow fell. Blood pressure was not affected. Skin temperature and heart rate were sharply decreased.

A single intravenous injection of atropine was given during infusion of pitressin in 7 experiments, without a definite effect on renal plasma flow. Inulin extraction percentage was moderately increased in most of the observations. The blood pressure rose about 40 per cent over the levels existing during the infusion of pitressin alone, while heart rate increased 2 to 300 per cent. Skin temperature remained unchanged.

Hypophyso-adrenal synergy and carbohydrate metabolism. E. L. COREY.

Department of Physiology, University of Virginia.

The pituitary gland has been shown by many workers to be associated with carbohydrate metabolism. Its correlated activities with other endocrine organs in this respect are, however, not distinctly understood.

The marked fall in blood sugar which supervenes in the hypophysectomized fasting animal has been clearly shown, in an earlier communication, to be ameliorated by extracts of the adrenal cortex. Further experiments have now demonstrated the definite ability of cortico-adrenal extract to maintain also a normal level of hepatic glycogen reserves in such animals. It was found indeed that in many cases even supernormal levels of liver glycogen were built up by the extract. Muscle glycogen was kept furthermore within approximately normal limits. Several series of rats were used, and treatment was given over a 24-hour fasting period. Large doses of extract, or small doses given frequently, were found effective.

Cortico-adrenal extract was also observed to bring about restoration of normal blood glucose and liver and muscle glycogen values in hypophysectomized rats which had been first fasted for 12 hours in order to deplete the carbohydrates in the body.

The replacement as well as maintenance function of cortico-adrenal extract in the regulation of carbohydrate metabolism, even after complete hypophysectomy, has thus been demonstrated. The operation of the hypophysis through the adrenal cortex, in its carbo-metabolic expressions, is also further attested.

Epinephrine output in experimental hyperthyroidism in dogs. RUTH CORTELL (by invitation) and J. M. ROGOFF. Department of Physiology, University of Chicago, Ill.

Six dogs were fed desiccated thyroid (Armour's, 0.2 per cent iodine content) by mouth daily in quantities ranging from 0.8 gram to 1.3 gram per kilogram of body weight, for periods ranging from three weeks to twelve and a half weeks. Observations were made for a control period previous to the thyroid feeding and were continued throughout the experimental period. Water intake and urine output were recorded daily; pulse rate, rectal temperature, respiratory rate, and body weight were recorded at frequent intervals. In one control dog and two of the experimental animals, basal metabolism was determined at intervals throughout the experiment, with the Benedict-Roth closed circuit apparatus.

All the dogs showed definite weight loss and increase in pulse rate; all but two showed increased water intake and urine output. Rectal temperature was elevated in some of the animals. The two thyroid-fed animals whose basal metabolism was determined showed average increases of 59 per cent and 51 per cent respectively, over the control observations.

At the conclusion of the experimental period, the epinephrine output from the adrenals was determined in each dog by the method of Stewart and Rogoff. In none of the thyroid fed dogs was the epinephrine output outside of the normal range as determined in the control animals, or in the large series of normal dogs studied by Stewart and Rogoff.

The effect of temperature on the electrical responses from the eyes of the grasshopper and moth. FREDERICK CRESCITELLI and THEODORE L. JAHN (introduced by J. H. Bodine). Zoological Laboratory, State University of Iowa, Iowa City. (Read by title.)

The electrical responses to illumination of the dark-adapted eyes of the grasshopper (*Melanoplus differentialis*) and of the moth (*Samia cecropia*) have been studied at temperatures from 0°C. to 50°C. Lowering the temperature from 25°C. to 0°C. causes a progressive increase in latent period, a decrease in magnitude, and a marked prolongation of the electrical response of the grasshopper eye. Similar treatment of the moth results, not only in increase in latent period, but also in a change in wave form. The b-wave is progressively decreased until it becomes indistinguishable; the a-wave is slightly affected in magnitude but its rate of development is lowered and its duration is increased. The c-wave is decreased in magnitude and rate of development as the temperature is decreased from 25°C. These changes are completely reversible.

Increasing the temperature results in an increase in magnitude of the grasshopper response between 25°C. and 35°C. and a progressive decline above 35°C. The wave form changes as the temperature is raised; the response becomes spike-like, and a small inflection which appears near the base of the descending limb of the response at 25°-30°C. becomes increasingly prominent. The a-wave, never present in the grasshopper response except with low intensities of illumination, may appear at high intensities above 42°C.

Marked changes in wave form of the moth eye response occur with increases in temperature above 25°C. The small, spike-like a-wave remains unchanged up to 30°C.; it then increases until it is greater in

magnitude than the prominent c-wave. As the a-wave increases, the b-wave becomes smaller and finally is indistinguishable. The c-wave, typically a prominent, slow wave at 25°C., increases in magnitude and rate of development as the temperature is raised to 30°C. and decreases progressively in magnitude above 30°C. At 42°-47°C. the response consists only of a fast, prominent a-wave and a relatively small c-wave. When this stage is reached, the changes, if reversible at all, are reversible only very slowly or incompletely.

Differential effects of curare in the central nervous system. E. A. CULLER.

The University of Rochester, N. Y.

Girden and Culler demonstrated that a conditioned pattern could be acquired, retained and revived by curarized dogs. The semi-tendinosus muscle of each hindlimb was exposed; either could then be readily conditioned to sound of bell in combination with shock to homolateral hind paw. The earlier observations may be summarized as follows:

| | SEMI-TENDINOSUS MUSCLES | |
|--------------|-------------------------|----------------|
| | Right | Left |
| Normal | CR established | |
| Curare | CR vanishes | CR established |
| Normal | CR reappears | CR vanishes |
| Curare | CR vanishes | CR reappears |

The following mechanism was hypothesized by Girden and Culler to account for these phenomena. The animal under curare is functionally decorticate (cortex depressed); what conditioning occurs is therefore sub-cortical in nature and locus, being inhibited when the cortex returns to dominance. Conditioning of the normal animal however is predominantly cortical in nature and locus, and therefore disappears when curare depresses cortical activity.

In this paper I attempt to test the validity of this inferential mechanism with evidence drawn from direct stimulation of motor roots and of cortex.

The animal is prepared by dissecting out the right M. semi-tendinosus, by exposing the ventral roots which innervate it (sixth and seventh lumbar, first sacral), and finally by laying bare the left motor cortex near cruciate sulcus. Stimulation was provided by a rectangular pulse of variable amplitude and duration.

Results: 1. Curare elevates *both* the cortical rheobase (excitation-time being constant) *and* the cortical excitation-time (current being constant).

2. Curare elevates neither rheobase nor excitation-time in the ventral roots; if anything they are slightly sensitized.

3. It follows that a *plane of cleavage* passes somewhere between cortex and ventral roots. On one side is a zone of depression; on the other a non-depressed area. How this effect is related to "dual personality" in man will be noticed.

Depression of gastric secretion by extracts of pregnancy urine. C. U. CULMER (by invitation), A. J. ATKINSON (by invitation) and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Based on reports that gastric secretion is decreased during pregnancy, that women with "peptic" ulcer gain relief upon becoming pregnant, and that A. P. L. would prevent and cure experimental "peptic" ulcer in dogs, experiments were performed to determine the effect of the pituitary-like fraction of human pregnancy urine upon gastric secretion. Five non-castrated female dogs, normal except for having Pavlov pouches, were the test animals. Two preparations of A. P. L. were used: *Antuitrin-S* (Parke Davis Co.) and *Follutein* (E. R. Squibb & Sons). Gastric secretion was stimulated by a standard test meal.

Eight experiments were performed on the five dogs, doses of A. P. L. varying from 1,000 to 5,000 rat units daily for five consecutive days being administered in each case. One dog showed a decrease too small to be considered significant. The other four dogs showed markedly significant depressions (of volume, free acid, and total acid); the experiment was repeated a second time on three of them with similarly significant results.

It was further found that the gastric-inhibitory factor was not inactivated by boiling for 10 minutes but was inactivated by boiling for 90 minutes. The A. P. L. sex hormone of pregnancy urine is damaged by heating to 60°C. and destroyed completely by boiling. The inhibition of gastric secretion is usually very prompt.

We conclude, therefore, that there is a substance excreted in human pregnancy urine which when injected subcutaneously or intramuscularly into dogs with Pavlov pouches has a marked inhibitory effect on gastric secretion. It is most unlikely that this substance is A. P. L.

Intercortical connections of the corpus callosum as indicated by evoked potentials. HOWARD J. CURTIS (by invitation) and PHILIP BARD. Department of Physiology, Johns Hopkins Medical School, Baltimore.

In monkeys under nembutal anesthesia single electrical shocks were applied to the cortex of one hemisphere and the other cortex explored for evoked potentials. The only regions not studied were those which cannot readily be exposed by removal of the calvarium. In general it may be said that localized stimulation of one cortex gives rise to distinct evoked potentials in one or more specific places on the other cortex. The magnitude of the response is quite variable, and may be as large as several millivolts. The wave form is also variable; it may be surface positive, surface negative or diphasic. The latency (measured from artifact to crest of the wave) varies from 10 to 40 msec.

The largest and most readily detected potentials are those obtained when the stimulating and recording electrodes are placed on symmetrically situated points of the two cortices. So far the only region which has not yielded potentials from symmetrical stimulation of the other side is the operculum of the occipital lobe (area 17). Localized stimulation within certain areas may evoke potentials over a considerable portion of the corresponding contralateral area. This is especially true of area 4 and the post-central gyrus. Also, judging by the evoked potentials an area on one side may be connected with portions of several areas on the other side. Area 7 seems to have especially widespread projections to the other cortex. In general a small displacement of the stimulating electrode will abolish or cause a shift in the place of origin of the evoked potentials.

These effects are completely abolished by section of the corpus callosum.

The birth process in the monkey as revealed by frozen sections. D. N. DANFORTH (by invitation), R. J. GRAHAM (by invitation) and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill. (Demonstration.)

Mounted (frozen) mid-sagittal sections made during the various stages of labor in 12 monkeys will be demonstrated. The specimens demonstrate the formation of the lower uterine segment prior to labor and the dilatation of the cervix, the drawing up of the cervix and "retraction," or brachystasis, of the upper and lower uterine segments during descent of the fetus.

*Antagonism of adrenalin to the autonomic effects of metrazol.*¹ CHESTER W. DARROW and ERNST GELLHORN. Institute for Juvenile Research and Departments of Medicine and Psychiatry, University of Illinois, Chicago.

In previous papers the writers showed that metrazol increases reflex sympathetic activity and that adrenalin may decrease reflex sympathetic responses by its peripheral action on homeostatic buffer mechanisms. Evidence is here presented that when the two drugs are given in various combinations the one may counteract the action of other.

Cats under urethane or chloralosane anaesthesia were connected for recording pupils, nictitating membranes (N. M.) blood pressure and galvanic responses of the foot pads. The sympathetic supply to one pupil and one N. M. was separated from the vagus and severed on one side in the neck, thus permitting comparison of the reflex responses with and without sympathetic supply. Photographic and kymographic records of responses to faradic stimulation of the central end of the sciatic nerve or brachial plexus were obtained. It is shown by slides:

1. That reflex response of the normal N. M., increased by metrazol, may be reduced or eliminated by intravenous adrenalin. N. M. responses suppressed by adrenalin may be restored by metrazol.

2. That the pupillary difference in reflex response of the normal pupil in excess of the sympathectomized pupil may be accentuated by metrazol and then reversed by adrenalin.

3. That reflex galvanic (sweating) responses of the foot pads may be reduced or eliminated by adrenalin and restored by metrazol.

4. That pressor blood pressure responses are reduced by adrenalin and increased by metrazol. This occurs usually in the presence of an increased level of systolic pressure in the case of adrenalin and of a reduction in pressure in the case of metrazol.

5. In the absence of metrazol, reflex sympathetic responses may be increased following ligation of the adrenals.

These effects are shown to be largely dependent upon the homeostatic action of the buffer nerves. It is attributable to the central action of one drug as contrasted with the peripheral action of the other.

Analysis of the electrical response of the human brain to auditory stimulation during sleep. H. DAVIS, P. A. DAVIS (by invitation), A. L. LOOMIS (by invitation), E. N. HARVEY and G. HOBART (by invitation). Department of Physiology, Harvard Medical School, Boston, and the Loomis Laboratory, Tuxedo Park, N. Y.

The electrical activity of the brains of human subjects was recorded during 25 afternoon naps. Definite electrical patterns regularly appeared as on-effects in response to tones of moderate intensity and frequency.

¹ Aided by a grant from The John and Mary R. Markle Foundation.

The responses ("K-complexes") were diffuse over the brain; largest in the central, prominent in the frontal, and lower voltage in the occipital and temporal regions. Similar "spontaneous" K-complexes may also appear in response to the sleeper's own breath sounds or without assignable external cause.

The K-complex is composed of two trains of waves, a fast sequence superimposed on a slow. The fast waves are more or less regular and rhythmic, usually between 8 and 16 per sec. Most characteristic in moderately deep sleep is a fairly regular 14-per-sec. rhythm similar to the "spontaneous" 14-per-sec. rhythm typical of this stage of sleep. The waves become slower and smoother as sleep deepens. In light sleep the fast waves are sharper and less regular, and resemble more nearly the alpha and beta waves of the waking pattern. Repeated stimuli at short intervals increase the fast activity and awaken the sleeper.

The slow component is a decelerating train of waves which first increase, then decrease in amplitude. The waves are slower, of higher voltage and form longer and more rhythmic trains as sleep deepens until they are indistinguishable from the spontaneous delta waves in the D and E stages. In the E stage no K-complexes can be evoked. The most prominent feature of the K-complex is a swing of 200 μ v. or more from scalp-negative to scalp-positive, occurring about 750 msec. after the onset of the tone. The slow component cannot be evoked for several seconds after a previous response or a "spontaneous" K-complex. The fast component then appears alone. The slow component appears to be closely related to the slow di- or triphasic on-effect of the waking state, and probably represents a higher voltage, prolonged, rhythmic activity of the same mechanism.

Depression of experimental polycythemia by choline hydrochloride. JOHN EMERSON DAVIS (introduced by Ray G. Daggs). Departments of Pharmacology and Biochemistry, University of Vermont, Burlington.

In 1938, the author presented work which showed that the feeding of raw beef or hog liver to polycythemic (experimental) dogs caused a prompt reduction in their erythrocyte numbers, by depressing hemopoiesis.

Recently, Jacobs (J. Lab. and Clin. Med. **24**: 128, 1938) has reported that liver extract, prepared for oral use, contains at least one per cent of choline.

This report led us to the investigation of the effect of orally administered choline hydrochloride upon polycythemic dogs. Polycythemia was produced experimentally in two dogs by placing them for six hours daily in a low pressure chamber in which the environmental air pressure was reduced to about 430 mm. of mercury by a motor driven vacuum pump. Within ten days, this procedure had induced consistent increases (27 and 30 per cent) in the erythrocyte numbers of the animals when in an approximately basal state. The hemoglobin percentage (Sahli) was increased correspondingly and reticulocytosis occurred. When 100 mgm. of choline hydrochloride per day was administered orally, prompt reductions (of 15 and 20 per cent) in the red cell counts occurred within three days, in spite of daily exposure of the animals to low atmospheric pressure.

The electrical response of the human brain to auditory stimuli. P. A. DAVIS (introduced by H. Davis). Department of Physiology, Harvard Medical School, Boston, Mass.

In 1935-36, 41 experiments were performed on 38 individuals in which

the response of the brain to tones was recorded by an ink-writing oscillograph. Electrodes were placed on the midline 2 cm. above theinion and at the vertex. (Reference electrodes were attached to the ear-lobes.)

Persons having alpha patterns and those with non-alpha patterns responded in a similar manner to loud and faint tones ranging from 250 to 2000 cycles. An on-effect, composed of a diphasic wave, appeared in both areas, though more prominently at the vertex. Often the response was triphasic, negative phase appearing first. Latencies could not be precisely measured but were about 30 to 40 msec. The total duration of the on-effect was approximately $\frac{1}{3}$ of a sec. or less. The voltage measured from the peak of the negative phase to the trough of the positive phase ranged from just visible to 100 μ v. Frequently there was an off-effect similar to the on-effect.

A response did not appear at all in some individuals. A checking of the alpha rhythm was sometimes the only response. The check would sometimes occur only once in a series of tones. An "anticipatory" on-effect frequently appeared at the appropriate interval after a regularly spaced sequence of tones was unexpectedly stopped. "Anticipatory" on-effects were also produced when regular intervals of tone were followed by irregular intervals. A "delayed" off-effect was produced by stopping the tone sooner than was expected. An "anticipatory" off-effect occurred when the tone was unexpectedly prolonged.

Conditions for varying the on- and off-effects are fundamentally similar to those for varying the response of the alpha rhythm to "eyes open" and "eyes closed." If drowsiness develops, the auditory on-effect is enhanced.

Studies on the "melanophore" principle of the pituitary. O. F. DENSTEDT (by invitation) and J. B. COLLIP. Department of Biochemistry, McGill University, Montreal, Canada.

Further investigation on the nature of the "melanophore" principle of the pituitary indicates that this factor is present in extracts in two forms. One of these apparently is less complex in character than the other since it dialyses readily through collodion and migrates at a more rapid rate on electrophoresis. The two forms differ also in solubility and in their stability to alkali.

A method has been developed by which the melanophore factors can be separated from the bulk of proteins in extracts. Studies made so far on the various fractions obtained in this separation suggest that the melanophore factors and the specific metabolic stimulant are not identical.

Electroencephalographic studies on irradiated animal brains. A. J. DERBYSHIRE and K. E. CORRIGAN (introduced by W. O. Nelson). Department of Anatomy, Wayne University, College of Medicine and Harper Hospital, Detroit, Mich.

The parietal cortices on one side of 12 dogs under dial or nembutal anesthesia were treated with dosages of 1700 to 5000 r units at 20 r per min. from the side portal of the 550 kilovolt x-ray machine at Harper Hospital. The beam was colimated to either 25 mm. or 12 mm. diameter and the target to brain distance was 60 cm. Electroencephalographic recordings with both unipolar and bipolar epidural leads showed no difference between the irradiated and non-irradiated hemispheres during the irradiation or for twelve hours afterward.

One parietal cortex in each of three dogs was treated with 25,000 r at 250 r per minute from the end portal of the same x-ray machine. Since the leads were implanted aseptically at the time of irradiation, records were taken from the unanaesthetized animal. The dominant labile 5 to 7 per second rhythm at 125 mv. persists until the 5th day from the treated cortex. Within the next few days this rhythm disappears from the irradiated cortex while it persists with reduced voltage from the non-irradiated cortex. During the 12 to 48 hour interval after irradiation brain wave patterns of the type associated with petit mal convulsions as well as one clinical seizure were observed. After a period of 3 to 11 days in which the animals are free of convulsions the brain wave pattern from both hemispheres showed frequent petit mal convulsions of about 15 seconds duration (slow waves 3-5 per second and spikes 19 to 30 per second and voltage of 600 mv.) accompanied by typical epileptiform seizures until three and one-half months after irradiation.

Vascular reactions in the nasal septum and skin of the head and digits.

JOHN B. DILLON (by invitation) and ALRICK B. HERTZMAN. Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

Vascular reactions have been simultaneously recorded in the finger, toe, and skin of the forehead, ear, and nose, and in the nasal septum with the aid of the photoelectric plethysmographs previously described. (A. B. Hertzman. Proc. Soc. Exper. Biol. and Med. **37**: 290, 529, 1937; Am. J. Physiol. **124**: 328, 1938.)

Spontaneous "vasomotor" waves may be seen in all areas but are usually more marked in the finger. In several instances, the nasal septum showed very large periodic waves independent of waves elsewhere. When present in the finger, they may or may not be present in the other areas. These waves are usually asynchronous in the different areas, suggesting origin in local vessel activity. They are frequently more marked after the cold pressor test or amyl nitrite.

Waves synchronous with respiration and partly due to movement are often seen in all areas.

The vasoconstriction in the digits following a deep inspiration or sigh is seen neither in head and ear skin nor in the nasal septum.

A strong psychic stimulus: marked constriction in the finger but not in the head skin.

Immersion of the hand in ice-water (cold pressor test); reflex constriction regularly but variable in extent in the finger, toe and nasal septum; either dilatation or no change in the forehead (a slight initial constriction was seen in 4 of 23 trials); similarly in the ear (one constriction in 23 trials); a variable response in the nose skin (no change, dilatation or constriction—few observations).

Inhalation of amyl nitrite: in finger and toe, usually constriction followed by a late dilatation, rarely an initial dilatation; in nasal septum, dilatation, sometimes preceded by an initial constriction; in forehead, ear and nose skin, marked dilatation, rarely constriction. The extent of the responses varies with the individual and the dose.

The results suggest: the skin of the head participates only weakly in vasomotor reactions; the reactions here are readily overcome by simultaneous changes in blood pressure (similar to the cerebral circulation);

caution is indicated in generalizing concerning cutaneous vascular reactions from observations on the extremities.

Further observations on the permanent diabetes produced by anterior pituitary extract. F. C. DOHAN (by invitation) and F. D. W. LUKENS. George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

Dogs have been made permanently diabetic by anterior pituitary extract as described by F. G. Young (1937). The urinary glucose and nitrogen during fasting has been compared with the findings in fasted normal and depancreatized dogs. The urine was sugar free in some animals, in others the glycosuria varied, occasionally reaching the level of the depancreatized dog. The urine nitrogen excretion varied from normal to depancreatized levels.

The feeding of fat resulted in a marked decrease in the excretion of glucose and acetone bodies.

Preliminary studies have been made of the respiratory metabolism in two dogs. The basal oxygen consumption was normal or slightly increased. There was a marked rise in oxygen consumption after meat feeding or after glucose and insulin. The respiratory quotient rose little or none after glucose.

Permanent pituitary diabetes has been produced in the cat (a relatively refractory species) by the removal of part of the pancreas before treatment with anterior pituitary extract. The results show that a smaller animal with an initially normal carbohydrate metabolism is available for these studies. They suggest an explanation for the species difference between the cat and the dog and indicate that the amount of pancreatic tissue plays a part in the susceptibility of species and of individual animals to the diabetogenic action of the anterior pituitary.

Studies on the elasticity of the aorta. PHILIP DOW (by invitation) and W. F. HAMILTON. Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta.

At the Baltimore meeting and in a recent paper, the authors reported experiments indicating that either direct stimulation of the intact right vagus or reflex depressor activity is accompanied by a slowing of the aortic pulse wave which is greater than can be accounted for by the lowering of the diastolic pressure. The present experiments are designed to identify the factors involved in this phenomenon.

A double cannula with recording tips separated by a fixed interval gives a constant distance (from just above the diaphragm to a point near the bifurcation) over which the passage of the pulse wave is timed at various diastolic pressures. The possible factors studied include injected epinephrine and acetyl choline, faradic stimulation of each vagus nerve intact, and stimulation of the central and peripheral ends of both vagi after cutting. It is believed that the phenomena studied may bear some relation to those recently reported by Wiggers, which involve active constriction of the aorta at high internal pressures.

In another series of experiments, the elasticity of the arterial reservoir has been analyzed from curves of pressure fall during prolonged occlusion of the aorta. Detailed measurement and graphical differentiation show that previous formulations based upon normal diastolic curves do not

represent the situation at lower pressures. Some of the discrepancies may be explained on the basis of an expected change in elasticity. Several long curves and extrapolations of others indicate cessation of outflow from the reservoir at pressures ranging from 18 to 26 mm. Hg. When the occlusion of the aorta is moved toward the heart, the shape of the curves shows that the drainage opened up is greater than the additional amount of reservoir included.

Localization of cerebellar cortical potentials in response to stimulation of various afferent connections. R. S. Dow (introduced by H. S. Gasser).

The Rockefeller Institute for Medical Research, New York City.

In contrast to a previous report (R. S. Dow, *J. Physiol.* **94**: 67, 1938), we are now able to obtain electrical potentials from the cerebellar cortex in response to stimulation of peripheral nerves. Single induction shocks or condenser discharges, rather than tetanic stimuli, have been used. Stimulation of the central ends of peripheral nerves of the upper or lower extremity in decerebrated or anesthetized cats resulted in responses in the anterior lobe of the corpus cerebelli, pyramis, lobulus simplex, and occasionally in the lobulus paramedianus. A response was obtained in the same lobes if the spino-cerebellar tracts were stimulated directly in the mid-thoracic region of the spinal cord. Except for a greater amplitude of response on the homolateral side, no indication of topographical localization of the upper and lower extremities was seen.

The vestibular nerve was stimulated by direct application of electrodes to the exposed nerve in the internal auditory meatus. Responses from this stimulation were found in the flocculus, nodulus, uvula and lingula and, in addition, in the fastigial nucleus. Isolated stimulation of other cranial nerves has been difficult, because of the ease with which reflex contractions of the neck muscles are produced. The question of the presence and location of responses to cranial nerve stimulation other than the vestibular requires further study.

Needle electrodes were placed in the pons and responses to stimulation here were found in the lobulus ansiformis, lobulus paramedianus, lobulus medius of Ingvar, in the paraflocculus, pyramis, lobulus simplex, and the culmen. The greatest responses were obtained in the hemisphere and in the central part of the vermis. Needle electrodes were similarly placed in the region of the inferior olive. Responses to stimulation were obtained in all parts of the cerebellum, but the potentials of greatest amplitude were found on the lobulus ansiformis. The potentials present on the lobes contralateral to the olive stimulated were the largest, but a slight homolateral response was seen as well.

Arteriosclerosis in depancreatized dogs. LESTER R. DRAGSTEDT, W. CARTER GOODPASTURE (by invitation), C. VERMEULEN (by invitation) and DWIGHT E. CLARK (by invitation). Department of Surgery, University of Chicago, Ill.

The abnormally high incidence of pre-senile arteriosclerosis in diabetes mellitus has been generally recognized. This defect has not been abolished by insulin therapy and constitutes one of the chief disabling complications of diabetes today. Varying degrees of arteriosclerosis of the aorta have been observed in six depancreatized dogs in our laboratory. The lesions have been found most frequently as small raised yellow nodules in the

intima near the orifices of the intercostal arteries and in the first part of the aorta near the base of the aortic valves. Microscopically, all degrees of involvement have been found from proliferation and thickening of the intima, deposition of amorphous lipoid material in the sub-intimal layers, and disruption of the media with deposition of amorphous and calcareous material in the media. For the most part, these animals have been maintained on mixed diets of protein, carbohydrate, and fat and adequate amounts of insulin, but with generally inadequate amounts of lipocaic. The hypothesis is suggested that these vascular lesions may be due to chronic lipocaic deficiency.

Additional experiments relative to the origin of glycoside emesis, using cats and dogs. M. DRESBACH. Departments of Surgical Research and Physiology, Medical School, University of Pennsylvania, Philadelphia.

This problem in the pharmacology of vomiting has never been solved. Some experimenters, e.g., Hatcher and collaborators—Weiss, Eddy, French (1922-1932), hold that the reflex is induced by the action of these substances in the heart; others, e.g., Hanzlik and Wood (1929), think that the liver or some other abdominal viscus is the seat of the action. It may yet prove to be central.

The aim here has been to supplement the work of Dresbach and Waddell (1926, 1928) by thoroughly interrupting, in surviving animals, the visceral afferent nerves of the heart, the abdominal organs and possibly of the heart and abdominal organs collectively. The following operations were done: cardiac denervation (4 dogs, operated by Dr. Norman E. Freeman, and 2 cats); complete bilateral vagotomy, just above the diaphragm (9 cats, 2 dogs); complete bilateral abdominal sympathectomy (2 cats, 2 dogs); bilateral abdominal sympathectomy, and double vagotomy above the diaphragm (2 dogs, 1 cat); transection of the spinal cord at different levels between T₂ and T₆ (4 cats); transection of the cord above T₅ plus double vagotomy and bilateral section of the splanchnics at level about T₁₀ or 11, in two separate cats.

Strophanthidin, ouabain, thevetin, K-strophanthosid and lanatosids A, B and C, in pure form chemically, were given intravenously and intramuscularly. Thus far, thirty experiments on twenty animals have been performed (in addition, some hepatectomies and liver denervations, Proc. Soc. Exper. Biol. and Med., 1936, 35, 92). Results: No matter how extensive the disconnection of the heart on the one hand and abdominal organs on the other from the central nervous system, there was no failure to excite the vomiting reflex by the above substances, providing good recovery had occurred from the surgical procedures and the animals were taking food normally. There was one failure in a dog, badly incapacitated accidentally by an injury to the medulla oblongata in a special experiment after cardiac denervation, and some cats in very poor condition, due to various post-operative complications, did not react. All animals were injected within four weeks of the initial operation; the study is being continued.

Further studies on aids to the absorption of insulin from the G-I tract. R. L. DRIVER (by invitation) and J. R. MURLIN. Department of Vital Economics, University of Rochester, N. Y.

Three classes of substances have hitherto been found effective in promoting the absorption of insulin from the G-I tract—alkyl derivatives of resorcinol^{1,2,3,4}, glycol derivatives⁵ and sapogenins⁶.

The present experiments were planned with the object of discovering: (1) the conditions under which each group acts best, and (2) which group under their respective optimum conditions would prove most consistent and dependable in this action. Acute experiments on amyotized dogs were chosen because many repeated tests can be carried out successively on one animal in the course of a day. To date of writing 26 dogs have been used in all-day runs.

The conditions for optimum effect would naturally include: (1) strength of solution; (2) pH; (3) the presence of various physiological or pharmacological substances known to affect the intestinal mucosa; (4) agents which combine with calcium and therefore remove this ion, known to reduce permeability, from the field of action.

The most satisfactory pH for the resorcinols is in the neighborhood of 9.5 to 10.5; for pinacol 3.7 to 6.5; for saponin a wide range whose optimum has not been found. Thiamin chloride seems to have a slight favoring effect on absorption; yeast is without effect. Digitonin, Strophanthin, ernutin, acetyl salicylic acid are all without effect. Calgon (sodium hexa-metaphosphate) favors absorption by combining calcium. This action is independent of the effect on pH.

The resorcinols and sapogenins are surface-tension lowering agents. Pinacol (tetramethylglycol) has very little effect on S.T. The resorcinols work best in dilutions of 0.125 to 0.5 per cent depending on pH; pinacol works from 1 $\frac{1}{10}$ to 5.0 per cent; strengths of 0.5 to 1 per cent are satisfactory for saponin.

Favorable effects seem to depend in part on surface action and in part on the presence of hydrophylic and hydrophobic groups. Of many additional compounds tried on the basis of these properties, di-octyl-sodium sulfo-succinate (Aerosol O.T.) has given best results.

The rate of disappearance of insulin from the body. D. R. DRURY and P. O. GREELEY (by invitation). Department of Physiology of the University of Southern California, School of Medicine, Los Angeles.

The fasting depancreatized dog requires insulin to maintain a normal blood sugar and the hourly insulin needed may be called its basal requirement. This has been found to be about 0.02 unit per kilo body weight per hour. If insulin be given at a higher rate than this glucose must be administered to maintain the animal in a steady state of blood sugar. These facts give us a basis for determining the effect of any given injection of insulin and we can measure the insulin activity in the animal at any time after administration. If the activity is high a large amount of glucose must be injected to keep the steady state; if the activity is low

¹ Murlin, J. R., R. L. Tomboulion and H. B. Pierce. *Am. J. Physiol.* **120**: 733, 1937.

² Daggs, R. G., W. R. Murlin and J. R. Murlin. *Ibid.* **120**: 744, 1937.

³ Murlin, J. R., L. E. Young and W. A. Phillips. *Science* **86**: 412, 1937.

⁴ Sealock, R. R., J. R. Murlin and R. L. Driver. *Am. J. Physiol. Proc.* **123**: 181, 1938.

⁵ Major, Ralph H. *Proc. Soc. Exper. Biol. and Med.* **38**: 721, 1938.

⁶ Lasch, F. and E. Schönbrunner. *Klin. Wchnschr.* **17**: 1177, 1938.

extra insulin must be given, and when the activity of a given dose has worn off entirely extra insulin must be injected at the basal rate of that animal.

We have followed this insulin activity on a series of depancreatized dogs after the injection of doses varying between 1 and 100 units, until the activity coming from the given dose becomes zero. The interval between injection and zero activity is the duration of action of that dose. On comparing the duration of action of different doses on the same animal we come to the conclusion that this time is not proportional to the size of the dose but is a simple function of the logarithm of the dose. If in a given animal 1 unit lasts 4 hours it will be found that 10 units will last 8 hours and 100 units about 12 hours. Such results would be expected if the insulin in the body is destroyed or eliminated at a rate proportional to the amount in the body at the time; stated mathematically $\frac{dx}{dt} = -kx$ where x is the amount of insulin in the body and k is a constant for each animal.

Additional evidence will be presented to support this contention.

Suppression of motor response upon stimulation of area 4-s of the cerebral cortex. J. G. DUSSER DE BARENNE and W. S. MCCULLOCH. Laboratory of Neurophysiology, Yale University School of Medicine, New Haven, Conn.

Late in 1934 we discovered the following phenomenon: the motor responses elicited by periodic repetitive electrical stimulation (a few seconds every minute) of a focus of area L.4 or A.4 of the macaque monkey is temporarily suppressed by stimulation (either electrically or by local strychninisation) of an area between L-A.4 and L-A.6a. This phenomenon has now been investigated more closely, the area from which it can be obtained carefully mapped and found to correspond to that area from which suppression of the electrical activity of areas L.4 and A.4 is obtainable, namely L.4-s and A.4-s (J. Neurophysiol. 1: 364, 1938).

Stimulation of area 4 itself or area 6a or both simultaneously (strychninisation) does not result in this phenomenon. This suppression of motor response has the surprising latency of one to several minutes. This phenomenon still occurs after an acute lesion of the nucleus caudatus or the pallidum and the sensory arm and leg nuclei of the thalamus. Therefore, it does not depend upon the one-way circuit L.4-s or A.4-s→nucleus caudatus→thalamus→L.4 and A.4 which mediates the suppression of the electrical activity of L.4 and A.4 upon local strychninisation of L.4-s or A.4-s (J. Neurophysiol. 1: 364, 1938), although an increase in motor response as well as in electrical activity of L.4 and A.4 ensues upon a lesion of the nucleus caudatus.

Total carbohydrate-acetone body utilization ratios with high acetone body concentrations. J. A. DYE and JANE L. CHIDSEY (by invitation). Department of Physiology, Cornell University, Ithaca, N. Y.

Total carbohydrate-total acetone body utilization ratios have been calculated for 28 nembutalized nephrectomized dogs given varying intravenous injections of Na acetoacetate. The total net carbohydrate utilization was determined, as glucose, from muscle and liver glycogen,

blood glucose, blood N.P.N. and blood lactic acid values—58 per cent of the protein metabolized was calculated as glucose. Ratios significantly above the theoretical 1:2 occurred, without exception, when the total acetone body concentrations were high, low ratios when they were low. These data indicate that the utilization of injected acetoacetate is a function of its own concentration in the blood and tissues and is independent of carbohydrate consumption.

In the following table, E8, E9, and E10 were eviscerated, all others non-eviscerated. In the former, glucose and lactic acid were considered to be distributed in $\frac{1}{4}$, total acetone in $\frac{1}{10}$ of the body weight; in the latter, glucose, lactic acid and N.P.N. in $\frac{1}{3}$, and total acetone in $\frac{1}{2}$ of body weight. Muscle was calculated as 50 per cent, liver as 3 per cent of body weight.

| DOG | BODY WEIGHT | GLUCOSE UTILIZATION | | Na ACETOACETATE UTILIZATION | | RATIO | ACETO-ACETATE INJECTED | AVERAGE BLOOD GLUCOSE |
|-----|-------------|---------------------|------------------|-----------------------------|------------------|-------|------------------------|-----------------------|
| | | mgm. per kgm., hr. | mM per kgm., hr. | mgm. per kgm., hr. | mM per kgm., hr. | | | |
| E8 | 12.5 | 401 | 2.23 | 432 | 7.45 | 3.34 | 589 | 200 |
| E9 | 12.0 | 489 | 2.73 | 605 | 10.4 | 3.8 | 722 | 52 |
| E10 | 11.5 | 459 | 2.55 | 728 | 12.6 | 4.9 | 807 | 94 |
| 6 | 7.5 | 205 | 1.14 | 247 | 4.26 | 3.74 | 355 | 67 |
| 7 | 7.4 | 341 | 1.89 | 283 | 4.92 | 2.6 | 582 | 65 |
| F1 | 10.5 | 387 | 2.15 | 658 | 11.3 | 5.2 | 1400 | 50 |
| F2 | 13.7 | 429 | 2.38 | 756 | 13.0 | 5.4 | 1456 | 56 |
| F3 | 6.8 | 318 | 1.77 | 483 | 8.3 | 4.7 | 1158 | 65 |
| F4 | 14.8 | 233 | 1.29 | 641 | 11.4 | 8.9 | 1016 | 63 |
| F7 | 13.6 | 239 | 1.33 | 304 | 5.24 | 3.9 | 618 | 60 |

The magnitude and time of development of the collateral circulation in occluded femoral and carotid arteries. RICHARD W. ECKSTEIN (by invitation) and DONALD E. GREGG. Department of Medicine, Western Reserve University, Cleveland, O.

Optical studies have been made of the magnitude and time of development of functional collaterals in the carotid and femoral arteries immediately after carotid or femoral ligation and also following chronic femoral artery ligation.

Within a few heart beats after femoral artery ligation the intrafemoral pressure peripheral to the point of occlusion fell to 20 mm. Hg with no evidence of a pulse, while the retrograde flow (of arterial blood) was 10 cc. per minute. Within approximately 30 seconds the peripheral femoral pressure showed a pulsation and the pressure and retrograde flow started to rise and became progressively greater. After approximately one hour further increases of pulse and flow were quite slow. At this time and with a good aortic pressure prevailing, 95 mm. Hg, the pulse was 110 and the retrograde flow 40 cc. per minute. When the femoral ligature was removed for 30 minutes and then re-applied the peripheral intrafemoral pressure dropped to 70 mm. Hg and retained a pulsation.

Essentially the same picture, although generally greatly accentuated, is found in the peripheral carotid artery immediately following its ligation.

Within 24 hours after the femoral artery was chronically ligated a good circulation had apparently been re-established in the leg peripheral to the point of artery occlusion, for the leg was quite warm and the dog used

it in a normal fashion. Measurement of the peripheral flow and pressure at this time and for weeks thereafter showed both pressure and flow to be considerably greater than those measured in the femoral immediately after its ligation.

The work done by muscles in walking. HERBERT ELFTMAN (introduced by W. O. Fenn). Department of Zoology, Columbia University, New York City.

The minimum amount of physical work which the muscles of the body must do in walking is equal to the fluctuation in the total kinetic and potential energy of the entire body for each step. An equal amount of energy is received by the muscles and disposed of as they are stretched under tension as the total energy decreases. The work actually done by the muscles will exceed the minimum if some of the muscles are being stretched while others are doing work. By instrumental recording of the external forces acting on the body, in conjunction with kinematic analysis of moving pictures, the torques exerted by the muscles on the parts of the body have been determined and the rates at which they do work obtained. The muscles which are properly situated for producing the torques required at any instant are not always so arranged that they will effect energy exchange in the same direction. If only one-joint muscles were present, the total work which they would have to do would greatly exceed the change in total kinetic and potential energy. The presence of two-joint muscles allows the actual muscular work to approach more closely the theoretical minimum.

Degrees of sterility in female vitamin E-low rats. GLADYS A. EMERSON (by invitation) and HERBERT M. EVANS. Institute of Experimental Biology, University of California, Berkeley.

Over a period of a decade or longer we have accumulated quantitative evidence of the need for increasing amounts of vitamin E as age progresses in order to restore fertility to E-low female rats. Such a finding, though supported with scanty data, has just been published by Barrie (Biochem. J. 32: 2134, 1938). From the end of the second to the sixth month of life, a single dose of a half gram of wheat germ oil will enable practically all E-low females of proved sterility to bear living young. At the end of the eighth month, with this level of wheat germ oil, three-fourths of such pregnancies terminate in resorptions. Yet higher curative levels of E at this time (2 grams) will result in the birth of living young in a third of the cases. Indeed, at the end of the first year of life, with four grams of wheat germ oil, almost a third of the pregnancies result in small litters of living young, though later it is probable that no amount of vitamin E will restore fertility in any case.

The effect of splenectomy on gonadotropic hormones. FREDERICK E. EMERY. University of Buffalo, N. Y.

Several reports have shown that continuous administration of gonadotropic hormones either as extracts or implants have in many cases given evidence of antihormone production. That the spleen may take part in producing these antihormones has also been considered. The present report gives evidence that in young rats the increase in the mean ovarian

weight following injection of extracts from the pituitary or pregnant urine is the same whether the spleen is present or absent.

The relative importance of toxemia in jejunal obstruction in dogs. C. A.

ENDER (by invitation) and R. C. HERRIN. Physiology Department, University of Wisconsin Medical School, Madison.

Loss of electrolytes with the consequent demineralization, dehydration, anhydremia, anoxemia and circulatory failure, has been demonstrated many times to be a very important factor in intestinal obstruction. The involvement of a nervous factor has been reported. In order to experimentally demonstrate toxemia the above mentioned factors must be absent and to provide conditions favorable for the development of toxemia, stasis of intestinal contents and intestinal distention must be present.

The above mentioned conditions seem to be satisfied by the following surgical preparation. The jejunum in dogs was divided 25-35 cm. from the ligament of Triest. Both ends were inverted by a purse string suture. The caudad end was anastomosed side by side to the lower portion of the duodenum or to the stomach. This results in a cecum-like loop of jejunum or duodenum and jejunum combined. About 10 cm. from the lower end of the obstructed bowel a pair of silver wires were placed in the wall of the bowel to make possible a fluoroscopic detection of the degree of distention. In 3 of 4 dogs prepared in this way vomiting resulted in little loss of electrolytes, apparently because the fluid from the obstructed loop found its way back to the normal bowel. One dog died on the 8th day of obstruction with a perforated bowel. One lived 2 weeks, another 6 weeks and another was apparently normal after 4 weeks. This type of obstruction was established in 2 dogs that had previously been denervated. One lived 16 days with no symptoms but died on the 17th day within a few hours of appearance of symptoms. The other showed no symptoms and appears normal on the 18th day of obstruction. Since the duration of life in all of these dogs excepting the one with a perforated bowel, was far beyond the 8 days reported for jejunal obstruction in dogs, the rôle of toxemia in acute obstruction seems to be of less importance than the chemical or nervous factors.

The influence of adult sex hormones on the differentiation of transplanted halves of embryonic genital primordia in the mouse. E. T. ERICKSON (introduced by J. H. Ferguson). Department of Zoölogy, University of Michigan, Ann Arbor.

In order to determine the plasticity of the embryonic reproductive system to sex hormones as they occur in the adult organism, mouse reproductive primordia of ten to fifteen days embryonic age, and gonads and ducts of sixteen to twenty-one days were implanted in a subcapsular position on the kidney of adult mice, one half into a male and one half into a female host.

Viable grafts containing ducts and gonads of both sexes were recovered after a residence of ten to twenty-five days on the host kidney. In no case could it be demonstrated that the original genetic sex of the gonad was altered, and no case of ovotestis was found. Effects attributable to the specific hormones were found in the accessory organs on histological examination.

The effect of certain drugs on the coronary blood flow of the trained dog.

HIRAM E. ESSEX, R. G. E. WÉGRÍA¹ (by invitation), J. F. HERRICK and F. C. MANN. Mayo Clinic and Mayo Foundation, Rochester, Minn.

Under ether anesthesia and sterile technic thermostromuhr units were placed on the right or the circumflex branch of the left coronary artery of the trained dog. After recovery from the operation the effect on coronary blood flow of various drugs injected into the saphenous vein, was determined. On the basis of this study the following statements are made: 1. Changes in blood flow in the right and left coronary arteries were qualitatively the same in response to the drugs given. 2. Drugs that lower blood pressure, viz: histamine, aminophyllin, nitroglycerine, M. A. P. (muscle-adenosine-phosphoric acid) and papaverine in appropriate doses increased the blood flow 100 per cent or more. The augmented flow was accompanied by an increased heart rate. 3. Among drugs that raise the blood pressure, *a*, pitressin decreased the flow in the coronary arteries as much as 80 per cent and lowered the heart rate; *b*, epinephrine increased the coronary flow 150 per cent or more with an increase in heart rate. 4. Atropine augmented the coronary flow as much as 70 per cent. 5. Coramine (pyridine-B-carboxydiethylamide) increased the coronary flow 100 per cent or more. 6. Anesthesia with nembutal (pentobarbital sodium) in one experiment caused a prolonged increase in coronary blood flow with a maximal increase of 60 per cent.

A direct result colorimeter. R. S. ESTEY (by invitation) and O. W. RICHARDS. Research Department, Spencer Lens Co., Buffalo, N.Y. (Demonstration.)

In a colorimeter of the Duboscq type the fundamental relationship between concentration and cup depth is stated in the equation:

Sample concentration = (std. concentration \times std. depth) / sample depth.

The fact that the sample depth appears in the denominator has led to result tables and graphs in which the relative concentration of the sample and the sample depth are related.

In the colorimeter to be demonstrated the millimeter scale numbering is abandoned and an arbitrary equal part scale is introduced which extends from 0 to 14.00 divisions in a depth range of 0 to 56 mm. This choice of scale interval places 10 near the center of the upper half of the range and makes the instrument direct reading from 0-140 per cent. In use the *sample* solution is set at 10.00 and the color match obtained by adjusting the *standard* solution depth. The result is evident by inspection of the scale on the standard side. Dividing the standard scale by 10 gives the sample as a fraction of the standard, or by multiplying the standard scale by 10 the sample concentration is expressed in per cent. For use with glass standards or with occasional concentrations for which a depth of 10.00 is unsuited, both scales may be read and the customary type of calculation used.

Other features contributing to the accuracy and convenience of the instrument will be demonstrated.

¹ Fellow of the Belgian-American Foundation.

The treatment of pneumococcic pneumonia with sulfapyridine. EVERETT IDRIS EVANS, TRACY D. CUTTLE (by invitation) and GARFIELD G. DUNCAN (by invitation). Medical Service "B", Pennsylvania Hospital, Philadelphia.

Fifty-five cases of pneumococcus lobar pneumonia have been treated with sulfapyridine. In almost all cases, a routine of Neufeld typing, blood cultures, frequent blood counts, serial x-rays, determination of concentrations of the drug in the blood stream, and daily physical examinations, has been carried out in this study. There has been noted clinical improvement in most cases within 24 hours after administration of the first dose.

Three deaths have occurred; all 3 deaths were in elderly patients, all were complicated by serious heart disease and decompensation, in two the pneumococcus was type III. Several toxic manifestations were noted: cyanosis, nausea and vomiting (common); hemolytic anemia, mild (frequent); leukopenia (rare, 1 case); the complication of empyema was noted in one case (inadequate treatment here).

One of us (T. D. C.) successfully treated one patient with pneumococcus meningitis. The drug was effective in one case of pneumococcus peritonitis, without effect in two cases of subacute bacterial endocarditis and one case of staphylococcus septicemia. There appeared to be no correlation between clinical therapeutic effect and blood level of the drug.

Sulfapyridine appears to be an effective drug in the treatment of pneumococcus pneumonia. Charts demonstrating typical therapeutic effects of the drug in the treatment of pneumococcus pneumonia will be presented. (A preliminary report.)

Preservation of the seminiferous epithelium and of fertility in male rats by prophylactic administration of alpha tocopherol. HERBERT M. EVANS, GLADYS A. EMERSON (by invitation), and OLIVER H. EMERSON. Institute of Experimental Biology, University of California, Berkeley.

Male rats were segregated at 21 days of age in such a manner that litter mates were in each of two groups of six animals. All groups received the standard E-low diet. Group I received daily orally from a calibrated dropper 0.75 mgm. of alpha tocopherol dissolved in 80 mg. of ethyl laurate. Group II received the solvent only.

After the fourth month, the well known decrease of growth rate was noted in all members of the E-low group as contrasted with those receiving alpha tocopherol. The growth rate of the alpha tocopherol group was identical with that characterizing normal animals on natural footstuffs in our colony. At the end of the fourth month of life, an estrous female was offered each male at approximately weekly intervals. On the following morning residual vaginal spermatozoa were sought in these females. In all cases of sexual congress the fate of the gestation was followed.

A marked contrast existed in the behavior of the alpha tocopherol and the E-low groups. Twelve matings of each animal in each group resulted in 521 living young of normal weight in the alpha tocopherol group, but no young were born in the E-low group.

All males were sacrificed when 277 days of age and careful dissection was made of the male reproductive system. The testes in the alpha toco-

pherol group averaged more than twice the weight of those in the E-low group (3.48 grams compared with 1.45 gram).

The epididymis in the case of each of the alpha tocopherol group contained abundant motile spermatozoa while none were found in the E-low controls.

Sections showed completely normal seminiferous epithelium in all tubules of the alpha tocopherol group. The Leydig tissue was normal or perhaps slightly less abundant than that sometimes found normally, an impression borne out by some reduction in the weights of the accessory organs of reproduction when contrasted with those of normal controls of the same age. However, no impairment of sex interest or of copulatory ability existed in the alpha tocopherol group inasmuch as in them we obtained 95 per cent of "positive matings", an average of 93 per cent being found in our general colony.

In the E-low group, all testicular tubules were degenerated.

Summary. Male rats on a standardized E-low dietary were fully protected from testicular degeneration by the daily administration of 0.75 mgm. alpha tocopherol.

The sequence of onset of injury potentials on the surface of the dog and turtle ventricle. J. A. E. EYSTER, WALTER J. MEEK, HAROLD GOLDBERG (by invitation), and H. L. BARTSCH (by invitation). Physiological Laboratory, University of Wisconsin, Madison.

There is strong experimental evidence to indicate that the positive potential occurring on the surface of an injured region of heart muscle (iap) is directly related to the activity of the surrounding muscle. Injury potentials produced by the suction electrode (H. C. Wiggers) used in a unipolar lead are remarkably consistent in form, sharp in onset and free from obvious action potential components arising from neighboring regions. Due to the charge distribution of the iap, the potentials are localized and affect neighboring regions to a negligible degree. These various characteristics would indicate the value of unipolar injury leads from regions of the heart surface as a measure of the relative times of onset of activity.

With the above considerations in mind we have measured the time of onset of the iap from various surface points of the dog and turtle ventricle as related to a standard reference curve. In the dog, the distribution closely accords with the results previously reported in which either unipolar action potential leads or the differential electrode was employed. The earliest onset of the injury potentials occur along the interventricular groove on the anterior and posterior surfaces. In general the surface of the right ventricle is involved earlier than the left, with the exception of the extreme right base and conus of the pulmonary artery. Most surface points are involved within 0.02 sec.

The work on the turtle ventricle was carried out on large specimens of the snapper, *Chelydra Serpentina*. The earliest onset of the iap in this species appears consistently on the surface of the lower mid ventricle, anteriorly and posteriorly and at the apex. The anterior surface near the left border up to the base is next involved and finally the mid and upper right base. There is little or no indication of a spread of activity in the form of a wave and widely separated points may be involved nearly simultaneously. In the exposed heart at room temperature all points are involved within about 0.2 sec.

The liver in relation to movements of potassium. WALLACE O. FENN.

Department of Physiology, School of Medicine and Dentistry, The University of Rochester, N. Y.

Eight rats were starved two days and were then fed a high carbohydrate diet for 24 hours. The liver was then found to be on the average 1.8 times as heavy as in four similar starved animals and it contained 12 per cent glycogen. Nevertheless the concentrations of water, potassium, chloride and acid soluble phosphate were substantially unchanged. These results indicate therefore, that when glycogen is deposited in the liver it is accompanied by appropriate amounts of water, potassium and phosphate and that as the liver enlarges, its extracellular chloride space enlarges likewise in the same proportion.

On stimulation of the leg muscles more potassium is lost than can be accounted for in the blood plasma or the total extracellular spaces of the body. Hence it must have penetrated into some cells. Resting muscles of the forelegs were analyzed for potassium and water before and after the stimulation of the hind legs but no increase in potassium content could be discovered except for a small increase when all the viscera had been previously removed from the animal. This evidence suggested the liver as one possibly important depot for potassium. For this reason the liver was analyzed for K, H₂O, and Cl (1) before and after a control period of 1 hour; (2) before and 1 hour after the intravenous injection of potassium in sub-lethal doses and (3) before and 1 hour after stimulation of muscles of the hind legs for 30 minutes. In 18 control cats the liver appeared to have lost chloride or extracellular fluid. This simulated a decrease in water and an increase in potassium content. After muscle stimulation in 12 cats the liver apparently lost 4.5 per cent of its weight as extracellular fluid but gained an equal amount of intracellular fluid containing 176 mM potassium per l. Thus 38 per cent of the potassium lost from the muscles could be accounted for in the liver. After potassium injection in 14 cats the liver lost 0.9 per cent of its weight as extracellular fluid but gained 1.9 per cent of its weight as intracellular fluid. Thus the liver, comprising 3 per cent of the body weight, absorbed 12 per cent of the injected potassium. The liver seems to be somewhat more efficient than other parts of the body in absorbing excess of potassium and has perhaps a certain importance from this point of view in muscular exercise.

The control of uterine motility in the puerperal rabbit. J. K. W. FERGUSON.

Department of Pharmacology, University of Toronto, Canada.

Since it has been demonstrated (Haterius and Ferguson. *Am. J. Physiol.* **124**: 314, 1938) that stimulation of the pituitary stalk in the puerperal rabbit releases an oxytocic hormone into the blood stream, an attempt has been made to find reflex influences that may affect the liberation of the hormone. It was suspected that dilatation of the uterus, cervix or vagina might provide an adequate stimulus for the release of the hormone and that this might be recognized by a characteristic, prolonged increase in frequency and amplitude of uterine contraction. In certain cases such a response was obtained by all three types of stimulation but failed to appear when the pituitary stalk or posterior lobe was destroyed. More often, however, it was impossible to distinguish such a response from a progressive tendency to increased activity, due probably to progressive diminution of anaesthesia. Dilatation of the vagina during light anaes-

thesia regularly gave a response not dependent on the pituitary. This was a "bearing down" reflex accompanied by a brief increase in the frequency of uterine contractions or an increase followed by a temporary inhibition. This response of the uterus resembled the effect of sympathetic stimulation or of epinephrine.

The high level of continuous uterine activity characteristic of the puerperal rabbit under light anaesthesia (chloralose and urethane) seems to be dependent on a continuous discharge of oxytocin since destruction of the pituitary leads to a progressive decrease of frequency and amplitude of the contractions. This continuous secretion appears to be dependent upon nervous impulses from the pelvic viscera, because severing the nervous connections, either at the pituitary stalk or just above the first lumbar vertebra, also causes progressive decline of uterine activity. That the impulses are not necessary for the formation of the hormone is demonstrated by the fact that electrical stimulation of the pituitary stalk, two or three hours after transection of the cord, gives even greater augmentation of uterine activity than it did before the section. This suggested that a considerable amount of oxytocin had accumulated during the period of quiescence.

A stereotaxic instrument for placing stimuli or lesions in the brain. J. K. W. FERGUSON. Department of Pharmacology, University of Toronto, Canada. (Demonstration.)

The skull is oriented by fixing three points; the two auditory meati and the space between necks of the central incisors. The advantages of this apparatus are its simplicity and speed of adjustment and its adaptability to skulls of any size or shape, ranging from rats to dogs.

The rôle of proteolytic enzymes, phospholipids, and tissue extracts in blood coagulation. JOHN H. FERGUSON and B. NIMS ERICKSON (by invitation). Departments of Pharmacology and Pediatrics, University of Michigan, Ann Arbor.

While it is an established fact that trypsin can clot citrated plasma and can activate prothrombin preparations *sans* added calcium, it has hitherto been overlooked that the enzyme is much more active in the presence of ionized calcium and that excess of citrate can inhibit its action.

These data completely re-orient our viewpoint as to the *modus operandi* of the enzyme in clotting. Instead of being an experimental curiosity, it now falls into line with the classical process of thrombin formation which is recognized to be a function of the calcium ion. It is necessary, however, to revise the view that only diffusible calcium ions are active, in favor of the hypothesis that ionization may occur at colloidal surfaces where the close juxtaposition of phospholipid and prothrombin permit of the thrombin interaction even in the presence of moderate amounts of citrate, etc.

In an artificial system consisting of *boiled* prothrombin, cephalin and calcium salts, there is no need to postulate an enzyme factor. However, the great thromboplastic potency of the phospholipid when presented in "free" solution as compared with the inactivity of the "combined" cephalin demonstrated in prothrombin by chemical analysis, suggest that the enzyme mobilizes not only the calcium but also the phospholipid. Added cephalin assists the trypsin action slightly.

The use of a quantitative method of comparing the thromboplastic activity of lung extracts and their extracted phospholipids with a standard

cephalin solution leads to the conclusion that this clotting function of tissue extracts is resolvable into *a*, a minor fraction, able to resist boiling, which is due to the phospholipid that can be "freed" by physicochemical means, and *b*, a major component, thermolabile, which is enzyme-like in character.

Minimal amounts of trypsin synergized by the presence of ionized calcium often yield clots which do *not* undergo fibrinolysis in a twenty-four hour period at 38°C. The correlation of coagulation and fibrinolysis gains support from the enzyme theory. A defect in the enzymic mobilization of the plasma phospholipid offers a plausible explanation of hemophilia.

The effect of faradic and galvanic stimulation upon atrophying skeletal muscle. ERNST FISCHER. Department of Physiology and Pharmacology, Medical College of Virginia, Richmond.

Excitability, weight, water content, resting O₂-consumption, and birefringence were determined for normal and denervated gastrocnemii of adult rats subjected to daily strong faradization or galvanization of one hind leg. The change in excitability of denervated muscles is characterized by diminished rheobase and by progressive lengthening of the chronaxie. At an early stage of atrophy, faradization lengthens the chronaxie still further during the first days of treatment. However, with prolonged faradization the chronaxie lengthens much more slowly than in the untreated denervated muscle. Thus, finally the excitability of the treated gastrocnemius will be higher than that of the untreated, although nevertheless lower than that of a normal muscle. At that time the loss in weight and in relative dry weight is already markedly diminished in comparison to the loss in an untreated muscle. Faradization starting only about ten days after denervation has only little beneficial effect. But in the later state of atrophy, galvanization will retard further atrophy and delay further loss of excitability. Galvanization directly after denervation has little or no beneficial effect. Combination of early faradization with late galvanization give the best results. Although atrophy cannot be prevented, the loss in weight, in relative dry weight and in excitability can be minimized or markedly delayed.

The resting O₂-consumption *in vitro* cannot be used as an index for the degree of atrophy and its arrest, since a) the metabolism increase in an untreated denervated muscle varies markedly, and b) faradization and especially galvanization does not decrease this excess metabolism, but tend to increase it more than proportional to the simultaneously observed increase in relative dry weight.

The submicroscopical structure of the atrophying muscle shows only moderate changes after 2 to 3 weeks. The observed decrease in total birefringence is much more due to a decrease in form birefringence than in micellar birefringence. These findings might be explained by assuming that during atrophy the relative volume of the micellae diminish and that their crystalline structure is impaired. Successful electric treatment also retards these birefringence changes to a certain extent.

The sensitivity of the oxygen consumption of yeast to cyanide. KENNETH C. FISHER (introduced by Laurence Irving). Department of Biology, University of Toronto, Canada.

Though in general the inhibition of the oxygen consumption of a given

cell is more or less constant, there are several instances where marked changes in cyanide sensitivity have been observed. The mechanism by which the variation of the cyanide sensitivity is produced is not established and indeed few of the characteristics of the change have been recorded. We have accordingly made experiments on yeast where the cyanide sensitivity decreases as a result of starvation, with the following results.

As determined by the direct spectroscopic examination of reduced cytochrome in unsaturated yeast (component *C* particularly) it appears to be quite possible to inhibit the Warburg-Keilin system practically completely though the oxygen consumption under the same circumstances is perhaps only 30 per cent inhibited. In saturated yeast however the respiration is 99.5 per cent inhibited.

The addition of paraphenylenediamine, which is a rather specific artificial substrate for the W.-K. system, to unsaturated yeast results in a seventy fold increase in oxygen consumption at least 97 per cent of which is prevented by cyanide.

The addition of dextrose to unsaturated yeast very rapidly (two or three minutes) restores the oxygen consumption to the value characteristic of saturated yeast. The cyanide sensitivity of such "saturated" yeast is initially low and thus similar to that observed in unsaturated yeast. It increases rapidly however, and after two or three hours at 20°C. has reached the value associated with saturated yeast.

These observations suggest that while a marked change in the affinity of the W.-K. system for cyanide certainly occurs, it is also necessary to suppose the presence of some system other than the W.-K. one through which oxygen is preferentially passed in unsaturated yeast. Moreover the mechanism producing the shift from one to the other of these systems requires an appreciable interval of time for its completion.

Spinal reflexes in nicotin poisoning. F. E. FRANKE. Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

The apparently contradictory reports in the literature on the action of nicotin on the spinal cord are probably largely due to the conditions under which the experiments were carried out. General anesthesia was usually used. The species of animal employed and the dose of nicotin given often were very different. In an attempt to study spinal reflexes under more nearly normal conditions, local or no anesthesia was used. Plaster of paris casts were often used to immobilize the animal and in some experiments an inflated blood pressure arm cuff was used to block the circulation to an extremity, thus protecting both receptor and effector. The nerve trunks are believed to be relatively resistant to nicotin. Thus the changes in reflexes could be attributed to a central action.

Experiments were first carried out in the normal animal without occluding the circulation. When sublethal or small doses of nicotin were given, the flexion reflex generally persisted throughout or was abolished for a shorter period of time than the knee jerk. However when large doses of nicotin, often many times the minimal fatal dose, were administered the flexion reflex and the knee jerk disappeared quickly and together. Respirations continue after sublethal doses and after large doses of nicotin fail later than the knee jerk and flexion reflex. The respiratory movements of the chest when present prove that all functions of the spinal cord are not paralysed. When artificial respiration is given spinal reflexes often

persist or return at a time when they would be abolished if this procedure had not been employed.

The occlusion experiments offer difficulties due to the great rise in blood pressure following nicotin injection and to the fact that the ischemic extremity will ultimately fail to function. Some evidence for central failure has been obtained with large doses, but further controls as to the completeness of circulatory occlusion are indicated.

Effect of progressive sympathectomy on hypertension produced by increased intracranial pressure. NORMAN E. FREEMAN and WILLIAM A. JEFFERS (by invitation). Harrison Department of Surgical Research and the Robinette Foundation, University of Pennsylvania Medical School, Philadelphia.

The intracranial pressure was raised in anesthetized dogs (nem-butal) by intracisternal injection of sterile physiological salt solution at 200 mm. Hg pressure. The blood pressure was recorded from the femoral artery by means of a Hamilton manometer. In normal dogs the systolic pressure increased to over 300 mm. Hg and the diastolic increased from 120 to 180 when the cisternal pressure was raised to 200 mm. Hg. The dogs were then allowed to recover before being subjected to progressive sympathectomy under aseptic precautions in successive operations under ether anesthesia. They were tested again after each stage of the sympathectomy.

Sympathectomy of the splanchnic area by lower thoracic ganglionectomy (T9 to T13) or by upper lumbar ganglionectomy (T13 to L2) and splanchnicectomy did not prevent the development of hypertension.

Upper thoracic sympathectomy (T1 to T6) did not prevent the rise in blood pressure from increased intracranial pressure.

When both upper thoracic (T1 to T6) and lower thoracic (T9 to T13) sympathetic ganglia were excised the hypertension from increased intracranial pressure failed to appear. This observation has been confirmed in four dogs. They are being tested each month. No recurrence of hypertension was found up to 107 days.

Hypertension produced by increased intracranial pressure was slight when the heart was denervated and the adrenals removed, even though the splanchnic sympathetic innervation was intact.

Extensive sympathectomy (T5 to L5) was performed on one dog. The major portion of the vascular bed was sympathectomized by this procedure but the cardiac nerves were left intact. Four days after the last operation (left, T5 to T13), hypertension did not occur when the cisternal pressure was increased. Sixteen days after the last stage, the systolic blood pressure went up from 160 to 240 mm. Hg. under the conditions of the experiment.

Conclusion. Sympathetic cardiac innervation, neural or humoral, is necessary for full development of hypertension produced by increased intracranial pressure.

The action of the dioxane derivative 933 F upon the blood pressure and heart rate of the normal and hypertensive dog. L. FRIEDBERG (by invitation) and L. N. KATZ. Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

There is a paucity of information concerning the hemodynamic effects

of drugs on the normal and hypertensive unanesthetized dog. We have developed a technique by which such information can be obtained. Dogs carefully selected and trained for a period of weeks were used so that readings on different days revealed a diastolic pressure variation of less than ± 5 mm. Hg. The blood pressure and heart rate were determined from records with direct femoral artery puncture employing the Hamilton needle manometer.

It occurred to us that if the chemical mediator responsible for the production of hypertension in the Goldblatt dog was sympathetico-mimetic in character as some have claimed, then an agent which is known to reverse this action should have a greater effect in the hypertensive than in the normal animal. The dioxane derivative (933 F), piperidomethylbenzodioxane, is such a substance. It reverses the epinephrine effect from a pressor to a depressor one in the anesthetized dog.

The dioxane derivative (933 F) was therefore injected intravenously in trained normal and hypertensive dogs in doses varying from 1 to 5 mgm./kilo and its effect on the circulation, and its ability to reverse the epinephrine pressor action compared. It was found that 933 F had a depressor action of equal degree in both the normal and hypertensive animal, and that this was accompanied by a marked tachycardia, a decrease in pulse pressure and by profuse salivation, shivering and pupillary dilation. In both normal and hypertensive dogs, 933 F had an equal effect in reversing the epinephrine¹ action from a pressor to a depressor one.

Our results, therefore, do not favor the view that the chemical mediator in renal hypertension is a sympathetico-mimetic substance like epinephrine. Since the pressor action of renin is not reversed by 933 F in either normal or hypertensive dogs, renin cannot be excluded as the possible chemical mediator of hypertension of renal ischemic origin.

*Induction of estrous behavior in anestrus cats with FSH and LH.*² HARRY B. FRIEDGOOD. Department of Physiology, Harvard Medical School, Boston, Mass.

Estrous behavior can be induced in spayed or anestrus females by injections of an estrogen alone, but the administration of an estrogen followed by injections of progesterone is more effective. Because of the well-recognized pituitary-ovarian relationship it seemed pertinent to inquire into the possibility of arousing estrous behavior in anestrus animals by administration of a purified anterior hypophyseal gonadotropic hormone. The extract used in these experiments was prepared by Dr. H. L. Fevold and consisted of a highly purified fraction of the follicle-stimulating hormone (FSH) which contained traces of luteinizing hormone (LH). Five of 6 anestrus young adult female cats which were injected daily with this preparation came into heat within 7 to 13 days. The total dosage used in each instance was 22, 34, 52, 77, 84 and 109 rat units of FSH. The cat which received 109 units for 16 days did not come into heat, and when autopsied was found to be a surgical castrate. The behavior of the estrous cats before, during and after mating was identical

¹ Incidentally, the action of epinephrine and renin was essentially alike in both the normal and hypertensive animal.

² Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

in every respect with the behavior of cats which come into heat spontaneously. The ovaries of these cats contained normal estrous follicles. Ovulation had occurred in two of the ovaries, and extensive luteinization of follicles without ovulation was also noted.

One may conclude that a highly purified fraction of FSH containing traces of LH induces estrous behavior in anestrus cats probably by means of ovarian stimulation.

The influence of glucose administration on gastric secretion. M. H. F. FRIEDMAN (introduced by T. L. Patterson). Departments of Physiology, McGill University, Montreal, Canada, and Wayne University College of Medicine, Detroit, Mich.

The effect produced on gastric secretion by administration of glucose was studied in dogs with a Heidenhain pouch and with a gastric fistula and oesophagotomy. Secretion was stimulated by histamine, alcohol, and sham-feeding. When the stomach was at rest and not secreting, oral or intravenous administration of glucose alone provoked only a scanty secretion. Glucose given by mouth before the injection of histamine greatly suppressed the volume of secretion but did not affect the total output of pepsin (which was low). The longer the time interval between the glucose administration and the histamine injection, the smaller was the inhibitory effect of the glucose. Oral administration of glucose 30 minutes before introducing 200 cc. of 5 per cent alcohol by rectum diminished the volume of secretion but increased greatly the concentration and total output of pepsin. Glucose administered intravenously diminished only slightly the secretion obtained by sham-feeding but did not alter the concentration or total output of pepsin (which was high).

In acute experiments on cats and dogs under Nembutal anaesthesia, intravenous injection of glucose (15 to 30 cc. of 5.6 per cent per kilo) always increased the rate of gastric secretion stimulated by histamine if the vagi were sectioned but frequently inhibited the secretion if the vagi were intact. The concentration and total output of pepsin was increased by glucose given intravenously if the secretion was stimulated by the injection of histamine and acetylcholine together but rarely if stimulated by histamine alone.

The inhibitory effect of glucose when given by mouth on the volume of secretion, as compared with its usual augmenting effect when given intravenously, suggests the possible intervention of enterogastrone. Whether it is given by mouth or vein, glucose has a specific trophic effect on the secretion of pepsin. This trophic effect is most marked when the peptic cells are being stimulated sub-maximally (by alcohol) rather than maximally (by sham-feeding), and is not evident when they are not being stimulated at all (by histamine).

Gastric secretion in Necturus. M. H. F. FRIEDMAN (introduced by T. L. Patterson). Departments of Physiology, McGill University, Montreal, Canada, and Wayne University College of Medicine, Detroit, Mich. (Read by title.)

In the healthy adult *Necturus* gastric secretion is spontaneous and continuous. The total acidity is about 35 milli-equivalents per litre, most of the acid being "bound". The fasting secretion is practically devoid of

peptic activity (modified Mett method). Secretion can be stimulated by the presence of food in the stomach or intestine but is in no way influenced by either parasympathomimetic drugs or histamine. Secretion of acid juice is indirectly inhibited by adrenaline presumably through its visceral vaso-constrictor action. Although a strong motor innervation of the gastric musculature is present in *Necturus* (Patterson. *Am. J. Physiol.* **84**: 631, 1928; Friedman. *Trans. R. Soc. Canada* **29**: 175, 1935) the gastric glands are not under direct nervous control. In this respect the gastric secretory and motor mechanism of *Necturus* is similar to that of the Elasmobranch fishes (Babkin, Friedman and MacKay-Sawyer. *J. Biol. Board Canada* **1**: 239, 1935) but markedly different from that of the frog (Friedman. *J. Cell. Comp. Physiol.* **10**: 37, 1937). In the frog there is a nervous control of both gastric glands and musculature.

Apparently the muscles of the stomach came under nervous control sooner than the glands. The inability of autonomic nerve stimulation to elicit a gastric secretion in those species where histamine is also ineffective (the skate and *Necturus*) suggests the possibility that the effectiveness of nerve stimulation on the gastric glands may depend on the liberation of histamine or a histamine-like substance as a mediator (MacIntosh. *Quart. J. Exper. Physiol.* **28**: 87, 1938).

The permeability of the gastric glands to glucose. M. H. F. FRIEDMAN and J. L. IRVIN (introduced by T. L. Patterson). Departments of Physiology and Surgical Research, Wayne University College of Medicine, Detroit, Mich. (Read by title.)

The concentration of glucose in gastric secretion stimulated by histamine was studied in dogs and cats under nembutal anesthesia. Before the actual experiments the animals were fasted for 24 hours. The slightest trace of food in the secretion was considered to invalidate the experiment. The total reducing substance ranged from 35 to 42 mgm. per cent and non-fermentable reducing substance from 10 to 15 mgm. per cent. These were constant values and did not depend on the blood sugar concentration. Raising the blood sugar to 500 mgm. per cent or more by intravenous administration of glucose did not increase the amount of reducing substance present in the gastric juice. In this respect the gastric glands are similar to the cat's salivary glands. A study is being made to determine whether the permeability of the gastric glands to glucose is altered by insulin, adrenaline, and other drugs.

Progression of symptoms during the insulin treatment. J. P. FROSTIG (introduced by H. E. Himwich). Department of Psychiatry and Neurology, Albany Medical College, Union University, Albany, N. Y., and the Harlem Valley State Hospital, Wingdale, N. Y.

Clinical observations were made during 6,587 insulin treatments. Symptoms were noted every 15 minutes with regard to their intensity and duration. Only those symptoms which appeared in 20 per cent of the cases are recorded. In order to achieve comparable results, the time of the action of insulin was arbitrarily limited to 5 hours. A table is presented which correlates symptoms with their time of appearance after the injection of insulin and their probable sites of localization in the brain.

The table shows that the symptoms advance through the different phylogenetic layers effecting first the newest and progressing to the

oldest. Increase of the dose of insulin speeds this process while decrease of the dosage slows the sequence of the symptoms. Nevertheless, the order of the symptoms remains unchanged. An explanation of the constancy of this sequence of symptoms may be found in the experiments of Himwich and co-workers. The neurological disturbances may be a result of the gradual decrease of the supply of carbohydrate of the brain and, therefore, of cerebral oxygen consumption. Thus a decrease of metabolic rate has a differential effect separating the functions of the various parts of the brain.

*The aggravation of pancreatic diabetes by adrenal cortical extract.*¹ E. G. FRY (by invitation), C. N. H. LONG and H. B. RITTER (by invitation). Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven, Conn.

When young rats of 25 to 30 days are partially depancreatized a considerable proportion (30 per cent) ultimately develop glycosuria. This may be still further exaggerated by the daily oral ingestion of from 2 to 5 cc. of adrenal cortical extract per 100 grams of rat. On cessation of the administration of the hormone the glycosuria frequently falls to sub-normal levels and then slowly returns to the level before treatment. This last effect is no doubt due to the atrophy of the animals' own adrenals that follows the administration of such high quantities of cortical hormone (cf. D. J. Ingle, G. M. Higgins and E. C. Kendall. *Anat. Rec.* 71: 363, 1938). During the period of hormone administration there also occurs a marked loss in weight which is regained on stopping treatment.

This aggravation of the glycosuria of partially depancreatized rats is superficially similar, with the exception of the change in weight, to that produced by anterior pituitary extracts but our recent experience has indicated that the mechanisms involved in the two cases are not necessarily the same. Thus, when adrenalectomized depancreatized rats are maintained at a constant level of glycosuria by daily oral ingestion of adrenal cortical extract, the injection of anterior pituitary extract is still effective in enhancing the glycosuria.

Consequently, it would appear that the anterior pituitary can influence carbohydrate metabolism *a*, by the action of the adrenotropic hormone in stimulating the activity of the adrenal cortex, and *b*, through another factor (or factors) that exert their effect directly on the tissues.

The influence of carbon dioxide on the utilization of oxygen by certain species of fish in the Toronto region. F. E. J. FRY and E. C. BLACK (introduced by Laurence Irving). Department of Biology, University of Toronto, Toronto, Canada, and Swarthmore College, Swarthmore, Pa.

The influence of carbon dioxide on the utilization of oxygen by certain species of stream fish occurring in the Toronto district has been measured by the following technique. Fish are sealed in bottles containing water high in oxygen and charged with various concentrations of carbon dioxide allowed to die there of asphyxiation. Subsequently the water is analyzed for free carbon dioxide and dissolved oxygen. When the results are

¹ This work was assisted by grants from the Committee on Research in Endocrinology, National Research Council and the Fluid Research Fund, Yale University School of Medicine.

plotted as P_{O_2} against P_{CO_2} , all fish fall on regular but specific curves, which show that the O_2 in the water at the time of asphyxia increases with the pressure of CO_2 . The sensitivity of fish in the Toronto region is compared with the sensitivity of fish in Algonquin Park and discussed.

Relation between conditioned and unconditioned reflex: the factor of state of the organism at the time of stimulation and of prolonged experimental repetitions. W. HORSLEY GANTT. Pavlovian Laboratory, Phipps Psychiatric Clinic, Johns Hopkins University, Baltimore, Md.

These experiments were designed to show what effect a great prolongation of the daily experimental period from the usual 1 hour to 20 hours without rest would have on the strength of both the conditioned and unconditioned food reflexes. Such a study is necessary for theoretical considerations and because prolonged periods of experimentation are used in following the effect of drugs on the cortical responses.

The unconditioned salivary reflex remains constant in size over a long period (20 hours) of continuous repetitions at 5 minute intervals. Furthermore the state of hunger has very little significance in determining the size of the unconditioned reflex for any given day.

The change in the conditioned reflexes is much more complex. Mere repetition for a long period of the conditioned signals, properly spaced and reinforced as usual, has little effect on the intensity of the conditioned reflexes. In most dogs however, there is a gradual decrease of the conditioned reflexes owing to the satiation as the experiment progresses. If this factor is eliminated by using very small amounts of food there is less change in the conditioned reflexes.

The changes observed in the conditioned reflexes are lengthening of the latent period both motor and secretory, disappearance of the weaker conditioned reflexes with preservation of the stronger, complete loss of the conditioned reflex with satiation accompanied by full strength unconditioned reflex as long as the dog can be induced to eat at all.

Constitution plays a rôle in the effect of repetition on differentiation between signals. In an excitatory, hyperactive unbalanced animal the inhibitory conditioned reflexes become excitatory. In the more stable animals there was no marked loss of differentiation.

Thus a marked difference exists between the relation of the conditioned and the unconditioned reflexes to the state of the animal (hunger, "incentive," subcortical excitation). Constitution is a determining factor in the changes suffered by the conditioned responses resulting from prolongation.

The effect of stimulation on the fat and carbohydrate content of the gastrocnemius muscle in the phlorizinized rat. CHALMERS L. GEMMILL. Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Md.

Although there is considerable indirect evidence that fat may be used for muscular contraction, no direct estimation of this change has been reported for warm-blooded animals.

Rats were given single injections of 60 to 800 mgm. of phlorizin in olive oil per kilogram body weight. Quantitative tests for glucose showed that these doses caused marked losses of glucose in the urine. The rats

were subjected to experimental analysis from one to nine days after the injection and were starved for periods of one to six days.

Under nembutal anesthesia, the tibial nerves were dissected and cut on both sides. The left nerve was stimulated with break shocks for periods varying from 0.9 to 2.66 hours and at rates varying from 17 to 64 stimuli per minute. Nine resting control experiments gave differences between the left and right gastrocnemii in the per cent of total fat of -0.33 per cent to $+0.24$ per cent with an average difference of -0.07 per cent. In the stimulated group, the differences between the total fat content of the working and resting muscles averaged -0.09 per cent, with variations from -0.55 per cent to $+0.22$ per cent in 12 experiments. With the exception of one experiment, the differences were similar to those in the control group. Even after six days of starvation and phlorizin poisoning, the stimulated muscle did not show significant changes in total fat content.

Following these experiments, total carbohydrate determinations were made on gastrocnemii from phlorizinized rats. The average difference between the total carbohydrate content of the resting left and right gastrocnemii was $+0.02$ per cent, with a deviation from $+0.07$ per cent to -0.02 per cent in five experiments. Stimulated muscles from normal animals gave an average decrease of 0.13 per cent in total carbohydrate when stimulated at the rate of 68 shocks per min. Analysis of stimulated and resting muscles of phlorizinized and starved rats showed a similar average difference, -0.13 per cent, with variations between -0.25 per cent and -0.07 per cent. In all cases there was a definite decrease in total carbohydrate in the stimulated muscle. The lowest value for the total carbohydrate of the resting muscle from a phlorizinized rat was 0.33 per cent, while from a normal it was 0.90 per cent. The utilization of this amount is sufficient to carry out muscular activity under the conditions of these experiments and thus the need for fat was not accelerated to a measurable degree.

The effect of insulin on muscle glycogen in vitro. CHALMERS L. GEMMILL.

Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Md. (Read by title.)

Corresponding sartorii from frogs were shaken in Warburg vessels containing glucose-Ringer's solution buffered with phosphate and bicarbonate and in an atmosphere of 2.5 per cent CO_2 and 97.5 per cent O_2 . After periods of five to six hours of shaking at 15.9°C . glycogen determinations were made in the two sets of muscles. The average difference in percent glycogen between the two sets of muscles was -0.01 per cent with deviations from $+0.04$ per cent to -0.12 per cent in seven experiments. Following the control series, similar experiments were carried out with the exception that amorphous insulin powder (22 units to a mgm.) was dissolved in the Ringer's solution of one vessel. The amount of insulin present in the vessel in the majority of the experiments was 2.2 units. The glycogen content of these muscles after a five or six hour period showed deviations of $+0.23$ per cent to -0.21 per cent, with an average of -0.01 per cent in ten experiments. Only in the two experiments giving the extreme values was there any deviation from the normal control series. It is concluded that insulin has no effect on the glycogen content of frog's sartorii under the conditions of these experiments.

A similar series of experiments was carried out using rats' diaphragms. In some of the experiments glucose-Ringer's solution buffered with both phosphate and bicarbonate was used with an atmosphere of 5 per cent CO_2 and 95 per cent O_2 . In other experiments only phosphate was used with an atmosphere of oxygen. The vessels were shaken for three hours at 37.85°C . In the control series the differences between the glycogen content of sections of the same diaphragm averaged $+0.01$ per cent, with deviations of $+0.04$ per cent to -0.02 per cent in five experiments. However, when insulin was added to one vessel in concentrations varying from 3.39 to 7.7 units, there was more glycogen in the diaphragm exposed to insulin. This series gave average differences of $+0.17$ per cent, with variations of $+0.05$ per cent to $+0.36$ per cent in seven experiments. Therefore, insulin has a small but definite effect either in building up or in holding the glycogen in the rat's diaphragm in vitro.

Action of drugs on potentials of isolated frog brain. R. W. GERARD and B. LIBET (by invitation). Department of Physiology, University of Chicago, Ill.

The regular electrical rhythm in the isolated frog brain can be subjected to experimental analysis almost as conveniently as an action in isolated nerve. The following data were obtained in a study of the metabolic control of these cell rhythms and of the mechanisms of synchronization and spread; and will be interpreted from these viewpoints.

Cyanide (0.005 to 0.1 M) converts the normal 6 per sec. rhythm of the olfactory bulb into a regular or irregular one of higher frequency (30 per sec.) and lower amplitude. After 0.1 M cyanide, activity disappears in Ringer, to reappear in the cyanide.

Iodoacetate (0.004 M) likewise accelerates the normal rhythm to 15 per sec., leaving it usually regular. In addition, large ($200\mu\text{V}$) slow (0.3 sec.) waves of irregular but constantly repeated profile recur at regular intervals of some 10 seconds. These may be spreading waves. Lactate does not affect the rhythm in fresh brain but regularizes and partially restores the potentials after iodoacetate. The striking waves induced by nicotine are prevented by the simultaneous action of iodoacetate.

Caffeine (0.01–0.5 per cent) increases the regularity and amplitude of the neural rhythm and slows its frequency—eventually abolishing the ordinary spontaneous waves. Its striking action is to initiate powerful (up to 1 mV) diphasic waves of 0.2 sec. duration that originate mainly in the bulbs and spread over the entire hemisphere. These recur once in ten seconds to once a second, depending on concentration and time of action, and are followed by smaller satellite waves of diminishing frequency. A third wave, lasting 1 to 4 secs. and persisting when the others have disappeared, spreads at about 1 cm. per sec. The main diphasic wave travels 5 cm. per sec. at 20° . At higher temperatures (low, converse) more of these appear at shorter intervals, and each wave is briefer and travels more rapidly. Nicotine does not block these spreading waves but slows their movement.

Mechanisms of central integration of motor activity as exemplified in the respiratory act. ROBERT GESELL. Department of Physiology, University of Michigan, Ann Arbor.

The inspiratory and expiratory reticular cells activate the respiratory

muscles. Both are continuously driven and would fire continuously and at an even pace were it not for mechanisms interrupting and modifying these discharges. Two basic drives produce an electrotonic outflow of current at the axon hillock which fire the neuraxone rhythmically in direct proportion to the strength of the exiting current. One is self engendered depending upon the metabolic gradient, membrane permeability and polarization and ion concentration. Rise of temperature, excess CO_2 , and O_2 lack, increase the potential drop and therefore the frequency of firing. This constitutes central drive. Convergence of myriads of signals on each neuromembrane constitutes reflexogenic drive. Local negativities at the dendrites or dendritic end of the perikaryon increase the potential drop and augment the prevailing inherent nerve cell discharge. Negativities in the proximity of the axon hillock decrease the exiting current at the axon hillock and produce inhibition. Receptors thought to drive the respiratory act are therefore connected in our scheme to excitatory portions of the nerve cell (chemoreceptors, nociceptors, Golgi proprioceptors, pulmonary proprioceptors). Inhibitory receptors are connected with inhibitory portions (blood pressor receptors, muscle spindles). Alternate phasic interruption of the continuous firings of the inspiratory and expiratory cells is a heterogenous product of rhythmic changes in excitability, of rhythmic changes in unicellular acceleration, and of central reciprocal innervation. The slowly augmenting pattern of inspiratory discharge and the steady state pattern are inborn in the inspiratory and expiratory reticular architecture. Unicellular acceleration of firing in the inspiratory act is attributed to self reexcitation through the agency of recurrent fibers. Recruitment is attributed to collateral excitation of sister reticular cells. Signals arising in the chemoreceptors and pain endings exert continuous drives importantly controlling acceleration and recruitment. This is a dual inspiratory and expiratory drive. The inherent patterns of activity are reflexogenically modified by the periodic proprioceptive signals. Vagal proprioceptive signals drive both the inspiratory and expiratory muscles. Like the vagal signals, increasing Golgi signals support inspiration to meet increasing resistance. Decreasing inhibitory signals from muscle spindles augment contraction.

The transplantation from tissue cultures and by direct homologous and heterologous grafts of adrenal tissue to the eye of the adrenalectomized rat. GEORGE O. GEY (by invitation) and ARTHUR GROLLMAN. School of Medicine, The Johns Hopkins University, Baltimore, Md.

The transplantation of tissues into the anterior chamber of the eye has proven to be more suitable than the classical methods of grafting tissues. By this means heterologous as well as homologous grafts are successful (Report of International Cancer Research Foundation, p. 36, 1935; Science 88: 357, 1938). We have adopted this procedure by transplanting human fetal and adult adrenal tissues, rat and guinea pig adult tissues and tissue cultures of rat and human adrenal tissues to the eyes of adrenalectomized rats. By transplanting various parts of the dissected adrenal it has been attempted to elucidate the problem of the functional differences and potentialities for growth of the different zones of the gland. The cells of the adrenal (human as well as the other animals) were grown in tissue cultures over long periods. The cultures were transplanted into the anterior chamber of the eye of adrenalectomized rats and the subsequent

clinical course of the animals noted. In successful grafts a microscopic study of the eye was subsequently made. By modifying the type of tissue used in the original cultures and the composition of the medium in which the tissue was grown, attempts are being made to develop a procedure which may be of practical clinical value in grafting adrenal tissue.

Cortical frequency spectra in three dimensions. F. A. GIBBS and A. M. GRASS (by invitation). Department of Neurology, Harvard Medical School, Boston, Mass. (Demonstration.)

The manner in which the energy in the cortical frequency spectrum shifts under various conditions can be shown by arranging a series of spectra to form a three dimensional model. A method for obtaining the cortical frequency spectrum was described before this Society last year. A full description is to be found in the *Journal of Neurophysiology* 1: 521, November 1938.

The effect of variations in CO_2 , sugar, and O_2 have been studied, and models have been made using one of these variables as the third dimension. In some cases where changes are produced by an unknown variable, time interval is used as the third dimension, for example in sleep and in epileptic seizures.

These models support our contention that it is profitable to regard the electrical activity of the cortex as a continuum. When data, of the sort met with in the E.E.G., are arranged as a spectrum, the span of comprehension is greatly increased. Features which cannot be seen or are seen only dimly in the unanalyzed record stand out clearly.

High CO_2 shifts the energy in the cortical frequency spectrum to the fast side; low CO_2 shifts the energy in the cortical frequency spectrum to the slow side; the effect is most marked at normal or near normal values. High concentrations of sugar and high O_2 tensions shift the energy in the cortical frequency spectrum to the fast side, but only at critically high levels. Low concentrations of sugar and low O_2 tensions shift the energy in spectrum to the slow side, but only at critically low levels. In sleep energy is progressively shifted to the slow side, the deeper the sleep the greater the shift. In certain types of epileptic seizure energy is shifted to the fast side, in others to the slow side. The lack of constancy of the cortical frequency spectrum in epileptics can be demonstrated, and a drift toward the fast or slow side (depending on the type of region) is often evident several days or hours before the seizure.

Studies on rats following the administration of estradiol benzoate. H. GILDER (by invitation) and R. A. PHILLIPS. The Scripps Metabolic Clinic, La Jolla, Calif., and Department of Physiology, Cornell University Medical College, New York City.

Warren O. Nelson (personal communication) has found that the administration of estrin to male and female rats immediately after hypophysectomy results in the eventual deaths of the males, but not of the females.

Unoperated and hypophysectomized male and female litter mates were given estradiol benzoate (Progynon-B), 200 rat units per 100 gm. body weight, intramuscularly, for 5 days and sacrificed, after a 24 hour fast, with and without the administration of glucose by stomach tube. An equal number (6 in each series) of controls—animals not given the estradiol

benzoate—were sacrificed after comparable periods with and without glucose. Two weeks were allowed to elapse after hypophysectomy before the administration of the estradiol. Liver and muscle glycogen, intestinal absorption, blood glucose and daily water intakes were observed.

Liver glycogen values were significantly higher 3 hours after glucose in the normal females given the estradiol than in normal females not given the estradiol. In the other groups there was no significant glycogen differences between animals which did and did not receive the estradiol. In another series, hypophysectomized males and females were not sacrificed. However, they all died within 10 days after cessation of the estradiol administration; while unoperated rats and male rats with the pituitary stalk sectioned, treated similarly, did not die. These observations may explain the selective lethal effect of estrin in the rats observed by Nelson.

Fisher et. al. (*Diabetes insipidus*, 1938, p. 162) have suggested that estrin inhibits the diuretic principle of the anterior lobe of the pituitary. There was a significant decrease in the water intake of all animals—normal, stalk-sectioned, and hypophysectomized—receiving estradiol benzoate. The decrease was as great in hypophysectomized as in normals. Since Fisher et al. cite no experiments on hypophysectomized animals it would seem dubious that their conclusions on this point are warranted.

The electrical field on the surface of the active turtle ventricle. HAROLD GOLDBERG (by invitation) and J. A. E. EYSTER. University of Wisconsin Medical School, Madison.

Potential time curves from some thirty points, distributed evenly over the ventricular surface, are recorded by unipolar leads simultaneously with a reference curve from a constant surface contact. The QRST interval is arbitrarily divided into forty to fifty equal time intervals and the potential at each point on the ventricular surface measured for corresponding times. The potential of resting heart muscle is arbitrarily taken as zero. Potentials from each instant are plotted on a ventricular diagram and the electric field indicated in the form of equipotential lines. The hearts of large snapping turtles (*Chelydra serpentina*) were used, either in situ, or isolated at the center of a large conducting bath.

Examination of such diagrams for successive time instants during the QRST interval leads to the following conclusions: 1. Electrical involvement of the heart is practically simultaneous throughout, regions of both negativity and positivity appearing. 2. The first portion of the QRS interval shows an increase in the potentials, positive and negative, in all regions, with the same general distribution and with slight shifts in space of the regions of maximum potential. 3. Subsequent reversal of polarity in certain regions does not occur by motion of regions of positivity and negativity but by simultaneous reversals of polarity from the preceding state in several regions. 4. Reversal of polarity may not occur in certain regions such as the apex, which gives a monophasic, positive, R complex, or may occur twice in the R complex as in some other regions. No distinct separation exists between the end of the R complex and the start of the T complex.

These observations favor neither of the following heretofore advanced modes of involvement: 1, a continuous, well defined, wavelike spread of

regions of negativity or positivity; 2, a continuous and well defined movement of a train or trains of dipoles.

This work has been recently extended to the mammalian auricle.

The effect of glucose and insulin on the disappearance of alcohol from the blood in man. WALTER GOLDFARB, KARL M. BOWMAN (by invitation) and SAMUEL PARKER (by invitation). The Psychiatric Divisions, Bellevue Hospital and King's County Hospital, New York City.

Investigators from various laboratories have presented evidence indicating that the oxidation of alcohol *in vivo* may be catalyzed by the simultaneous oxidation of carbohydrate. Goldfarb and Bowman (1938) found that the oxidation of alcohol by hydrogen peroxide *in vitro* was accelerated when glucose was present. We are presenting observations on the effect of 25 grams of glucose injected intravenously, 15 units of insulin injected subcutaneously, and both glucose and insulin on the disappearance of alcohol from the blood in man.

Blood alcohol determinations were made on acutely intoxicated patients on admission, and again 2 hours after the therapy was instituted. Insulin alone had no effect, while glucose alone increased the rate of disappearance of alcohol only in those patients whose blood alcohol exceeded 300 milligrams per cent. This effect was not observed in the less severely intoxicated group. Therapy with both glucose and insulin accelerated the decrease of blood alcohol in all patients. Patients treated with glucose and insulin showed more rapid clinical improvement. It is suggested that the oxidation of alcohol may be catalyzed by the simultaneous oxidation of glucose.

Changes in blood alcohol following therapy

| THERAPY | CONCENTRATION GREATER THAN 300 MGM. % | | | | CONCENTRATION LESS THAN 300 MGM. % | | | |
|--------------------|---------------------------------------|-------------------|----------------|---------------------|------------------------------------|-------------------|---------------|---------------------|
| | No. of Exp. | Change in 2 hours | Difference | Prob. sig. of diff. | No. of Exp. | Change in 2 hours | Difference | Prob. sig. of diff. |
| | | mgm. % | mgm. % | per cent | | mgm. % | mgm. % | per cent |
| None..... | 8 | -54 \pm 2.0 | | | 18 | -48 \pm 2.3 | | |
| 15 U. insulin..... | 9 | -53 \pm 3.8 | +1 \pm 4.3 | 8 | 7 | -53 \pm 6.7 | -5 \pm 7.1 | 58 |
| 25 gm. glucose.... | 10 | -110 \pm 7.4 | -56 \pm 7.6 | 100 | 12 | -49 \pm 3.0 | -1 \pm 3.7 | 16 |
| Glucose-insulin.. | 15 | -167 \pm 9.7 | -113 \pm 9.9 | 100 | 10 | -86 \pm 7.4 | -38 \pm 7.8 | 100 |

Motor conditioning in the goat. MARVIN M. GOLDMAN (introduced by H. S. Liddell). Department of Physiology, Cornell University Medical College, Ithaca, N. Y.

The aim of this investigation is to produce the experimental neurosis in the goat through a procedure of motor conditioning which has been found repeatedly to precipitate the experimental neurosis in sheep, pig and dog. A rapidly beating metronome with electric shock to the left forelimb after 10 seconds is followed 5 minutes later by a slowly beating metronome for 10 seconds without shock. The daily test includes a sequence of positive, negative, positive and negative rates of the metronome each of 10 seconds duration separated by 5 minutes rest intervals. The following items of behavior are systematically observed and recorded throughout each experiment; movements of the head and left forelimb,

respiration, and the heart beat (employing the cardi tachometer). The effect of the daily conditioning upon the behavior of the animals outside of the laboratory is studied for evidence of premonitory signs of the neurosis. Of the four adult goats employed the male is considerably more excitable than the 2 castrated males and female as shown by a much more vigorous and diffuse conditioned reflex and extremely variable heart rate.

The influence of gum acacia on renal function. A. GOUDSMIT, JR. (by invitation), M. H. POWER and J. L. BOLLMAN. Divisions of Biochemistry and Experimental Medicine, The Mayo Foundation, Rochester, Minn.

Gum acacia has been found to be a valuable diuretic agent in certain clinical states associated with low concentrations of protein in the blood serum as well as in conditions of experimental hypoproteinemia. The reason for its administration obviously is the desire to increase the deficient colloid osmotic pressure of the serum of the recipients. However, actual measurements of the colloid osmotic pressure before and after therapeutically successful administration of gum acacia reveal no significant differences, nor can any other known effect of its administration definitely be considered as the direct causative factor of the increased diuresis.

Therefore the question was raised whether gum acacia might have a more direct influence on the function of the kidney. Experiments on normal dogs were performed, and, under standardized conditions, creatinine and urea clearances were studied in addition to the rate of excretion of water and chlorides before, during and after the intravenous injection of autoclaved solutions of gum acacia, in 5 per cent glucose. It was found that no significant changes occurred in the value of creatinine or urea clearances, however uniformly the rate of chloride excretion was increased. This increase ranged between 4 and 352 per cent, on the average 190 per cent, even though there usually occurred a drop in the concentration of chloride in the plasma, and appeared to be independent of changes in the urinary volume. This increase in chloride excretion appears to be related to the tubular components of renal activity and is considered significant since permanent reduction of edema is dependent upon the ability of the organism to dispose of excessively stored sodium chloride.

Effects of polarization on the action potential of C fibres. HELEN TREDWAY GRAHAM. Department of Pharmacology, Washington University School of Medicine, St. Louis, Mo.

Cathodal polarization decreases the spike potential of the response of C fibres (bullfrog splanchnic, cat hypogastric, vagus and cardiac nerves) and usually its early positive after-potential as well. Anodal polarization with currents of very few microamperes increases the positive after-potential, and this increase tends, at least in the multifibred nerves investigated, to obscure any increase of spike height that may occur simultaneously. With slightly stronger anodal polarization, increase of negative after-potential decreases the apparent positive after-potential, and with still stronger polarization (currents of 30 microamperes or so), the negative after-potential developed is so large as to obliterate the positive after-potential, and to raise the crest of the spike far above its normal level. That the spike potential is itself increased and not simply raised by superposition upon the concurrent negative after-potential is indicated by the

form of the response, but the anodal increase of spike height is certainly much less than in *A* fibre action potentials.

The effects of polarization on *C* fibre response differ from its effects on *A* fibre response also in the apparently opposite action on the early positive after-potential in the two cases. The increase of after-positivity with cathodal polarization of mammalian *A* fibres is however most satisfactorily explained not as actual increase of positive after-potential, but as removal of negative after-potential and consequent disclosure of positive after-potential already present. There is then no contradiction in the effects of polarization on the two types of fibre, and a slight increase of positive after-potential sometimes seen with weak cathodal polarization of *C* fibres indicates removal of negative after-potential from the response of these fibres also.

To the facts already known regarding the effects of polarization on nerve action potential, the findings with *C* fibres add the increase of early positive after-potential with anodal, and its decrease with cathodal polarization. This effect of polarization provides evidence for the direct relationship of the early positive after-potential to the spike potential suggested by Gasser, since it shows that these two potentials vary in size together, just as do the negative after-potential and the late positive after-potential related to it.

*The respiration of uterine tissue of the rabbit and immature rat.*¹ MARK GRAUBARD (by invitation) and GREGORY PINCUS. Biological Laboratories, Clark University, Worcester, Mass.

Over a period of 6 hours after excision the oxygen uptake of strips of rabbit uterus is constant and the absolute values of the QO_2 are the same in rabbit serum as in phosphate buffer at pH 7.4. The QO_2 is directly proportional to the dry weight of uterine strips of varying sizes below a maximum size of 500 milligrams. Mashed tissue shows no oxygen uptake but oxygen is consumed on the addition of paraphenylene-diamine. Addition of cytochrome increases the rate of uptake. Intact uterus does not oxidise paraphenylene-diamine and shows no increased O_2 uptake on addition of cytochrome.

The oxygen uptake of both uterine cornua and the cervix of 30 to 33 day old rats (weighing 45 to 60 gms.) declines with time, but is practically constant for the first $3\frac{1}{2}$ hours after excision. At six hours after the injection of oestrogen (cf. Astwood, *Endocrinology*, **23**: 25, 1938), the uteri increase in weight depending on the hormone dosage. The oxygen uptake of hormone stimulated uteri also increases. The QO_2 per gram of wet weight is higher in uninjected rats, but the QO_2 per gram of dry weight is lower in the uninjected series. This is due to the fact that the weight increase on hormone stimulation is largely caused by the imbibition of water. The QO_2 per gram of dry-weight in hormone treated animals is directly proportional to the hormone effect as measured by the ratio $\frac{\text{uterine weight}}{\text{body weight}}$. Injected oestrone, oestriol, stilboestrol, and certain urinary estrogenic extracts exert parallel effects on the rat uteri preparations.

¹ Aided by a grant from the Rockefeller Foundation.

*The presence of a gastric secretory depressant in normal urine.*¹ J. S. GRAY, E. WIECZOROWSKI (by invitation) and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The fact that commercial extract of pregnancy urine have been found in this laboratory to inhibit gastric secretion by a direct action on the gastric glands, suggested the possibility that urine might provide a source of enterogastrone, which would be relatively free of protein impurities. Since enterogastrone is precipitated from extracts of duodenal mucosa by tannic acid, this reagent was utilized in preparing material from human male urine. Material obtained by this method has been found to inhibit gastric secretion in doses equivalent to 100 cc. or less of urine. More potent preparations, however, have been obtained from both human and dog urine, by the benzoic acid adsorption technique of Katzman and Doisy. Further purification has yielded a material which is approximately 25 times as potent in inhibiting gastric secretion as the usual preparations obtained from duodenal mucosa. The active principle has been found to be stable to boiling for five minutes. It exhibits little or no effect on gastric motility, is free of vasodilator action, and possesses no gonadotropic activity. Although the chemical and physiological behavior of the active principle obtained from urine resembles that of enterogastrone prepared from duodenal mucosa, it can not be stated, as yet, that the two are identical.

The composition of gastric juice as a function of the rate of secretion. J. S. GRAY and G. BUCHER (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill. (Read by title.)

A total of 283 samples of gastric juice secreted continuously and uniformly in response to repeated injections of histamine have been collected from six dogs with pouches of the entire stomach. Determinations of HCl, Cl, neutral chloride, potassium, calcium, sodium (by difference) and freezing point depression have been made. A mathematical and statistical analysis of the data have revealed the following relationships.

a. The outputs of HCl, Cl, neutral chloride, and K bear a direct linear relationship to the volume-rate of secretion; the output of Ca and Na is constant.

b. The concentrations of HCl and Cl bear a direct, those of neutral chloride, Na, and Ca an indirect hyperbolic relationship to the volume-rate of secretion. The concentration of K is constant at 7 m. eq. per liter.

c. The maximal (asymptotic) concentration of HCl and Cl were found to be 160 and 170 mm. eq. per liter respectively; the minimal (asymptotic) concentrations for neutral chloride, Na, and Ca were found to be 7, 0, and 0 respectively.

d. The concentration of HCl bears an inverse linear relationship to the concentration of neutral chloride.

These results are most easily interpreted by assuming that gastric juice consists of two components, a parietal secretion, which contains 167 mm. eq. of Cl, 160 mm. eq. of H, and 7 mm. eq. of K per liter, and a non-parietal secretion, whose composition is essentially that of blood

¹ Aided in part by a grant from the Committee on Endocrinology of the National Research Council.

serum. The variations in the composition of gastric juice secreted in response to histamine can be attributed to admixtures of varying amounts of parietal secretion with a constant amount of non-parietal secretion.

The effects of crustacean eye-stalk-hormone on the melanophore size and water uptake in the frog. STEPHEN W. GRAY and WALTER E. FORD (introduced by F. R. Steggerda). Departments of Zoology and Physiology, University of Illinois, Urbana. (Read by title.)

Color changes in the crustacea have been shown to act on the melanophores due to a hormone elaborated in the eye-stalks. It has been demonstrated many times that either implantations of the pituitary gland or injections of extracts of the gland caused in the frog a marked dilatation of the melanophores and an increase in weight due to water imbibition. Therefore, it was of interest to find whether the eye-stalk of the crustacea had any similar influence when injected into the frog.

To do this, a comparative study was made of the effects of 1, pitressin (Parke-Davis); 2, vertebrate pituitary bodies (fish, frog and turtle), and 3, eye-stalks (Fiddler crab and crayfish) upon melanophore size and weight changes in the frog. The results show that in all cases there is both a melanophore expansion and weight increase in the frog following these injections. It was also demonstrated that quantitatively 1 cc. of pitressin had approximately the same influence as one vertebrate pituitary gland or twelve crustacean eye-stalks. Similar changes were demonstrated to take place in the hypophysectomized frog. These effects imply a similarity in the function between eye-stalk-hormone and the vertebrate pituitary.

Direct measurement of the phasic blood flow in coronary arteries. HAROLD D. GREEN and DONALD E. GREGG. Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O.

Coronary blood flow has been recorded by the differential manometric method demonstrated at this meeting, together with differential pressure curves (Am. J. Physiol. **112**: 362, 627, 1935). Comparison of the curves confirms the conclusions drawn from constant pressure flow meter studies (Gregg and Green, these Proceedings) that the differential pressure curves faithfully record the proper time relations and directional changes of flow but may underestimate the phasic variations of velocity. In addition the isometric retardation and the inflow during rapid ejection and isometric relaxation may be relatively greater than indicated by the differential pressures.

During elevation of systemic pressure (compression of the lower thoracic aorta or blood transfusion) or temporary reduction of coronary blood supply (flow measured after restoration of normal blood supply) the blood flow increased more than was indicated by the change of differential pressure.

Although a loss of pressure head in the blood stream of 3-4 mm. Hg occurs during rapid rates of flow due to the orifice, this is insufficient to appreciably affect the vascular bed since continuous use of the meter over long periods of time causes no alteration of the blood flow.

Supplementary apparatus for optical registration. HAROLD D. GREEN. Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O. (Demonstration.)

Manometer stand. A vertical steel post carries a horizontal triangular shaped support upon which the manometers are mounted. The animal board is fastened to a heavy horizontal rod attached to the post. Calibrating manometer, electrical switches, infusion bottles, etc., are also conveniently supported by the post.

Mirror amplifier. By reflecting the light beam from the manometer mirror to a stationary mirror mounted immediately in front of the manometer, then back to the manometer mirror and from it into the camera slot, double the excursion of the recording beam is obtained.

Differential manometer modification. The fluid in the anterior chamber of a differential manometer frequently exerts a prismatic effect on the light beam. This has been overcome by mounting the window (a plano-convex lens) in a ball and socket joint so placed that by tilting the lens can be made parallel to the mirror. This arrangement permits sharp focussing of light beams and eliminates the colored fringes.

Square root extractor. Use of the differential manometer with a Pitôt tube or orifice principle for measuring fluid flow gives a calibration curve in which the deflection varies proportional to the square of the flow. Calculation of the total flow in pulsatile streams requires laborious redrawing of the curves with ordinate values made linear. A mechanical device has been constructed which greatly simplifies this labor. It consists of two pointers which slide along a track at right angles to the time axis of the curves. One of the pointers contacts the periphery of a spiral shaped wheel, the movement of the other is controlled by a wire attached to the circumference of a circular wheel which is integrally fastened to the shaft of the spiral wheel. When the contours of a flow curve are traced with the first pointer the second pointer gives a corrected tracing in which the ordinate values have a linear relationship.

A meter for measuring small blood flows of pulsatile streams. HAROLD D. GREEN and DONALD E. GREGG. Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O. (Demonstration.)

Rate of flow is measured by directing the blood through an orifice (or Pitôt tube arrangement). The momentary acceleration of the blood so produced causes a lowering of the lateral pressure for a short distance downstream which is proportional to the square of the acceleration. The moment to moment difference between the lateral pressures above and below the orifice, recorded by a differential manometer indicates the phasic changes in the rate of blood flow.

The upstream lateral pressure is led to manometer tip with a 4 mm. opening covered with a special rubber membrane 0.006 inch thick stretched 3 to 5 times. A waterproof plane mirror (chip from a Bureau of Standards certified counting chamber coverslip) is mounted on the membrane by means of a small hard rubber peg or splint (to avoid hysteresis). The downstream lateral pressure is led directly to a watertight chamber which surrounds the membrane and is constructed of transparent Lucite. The light beam enters this chamber through a +0.5 plano-convex lens at least 1.5 mm. thick. A small angle prism, capable of rotation through 360°, mounted in front of the lens, corrects the prismatic effect of the Locke's solution with which the chamber and unit are filled. The differential manometer is mounted in the carriage of a Gregg manometer.

When carefully filled so as to avoid air bubbles the assembled meter has a frequency of 80 to 120 double vibrations per second and gives a deflection of 40 to 60 mm. at 4 meters for a differential pressure of 10 mm. Hg which is sufficient to detect flows as small as 10 cc. per minute. At flows of 60 to 80 cc. per minute the net loss of pressure head in the stream is not more than 3 to 4 mm. Hg. The meter faithfully follows the alternating flow produced by a reciprocating plunger and the sudden starting and stopping of the flow produced by a stopcock.

Disappearance rate of tyrosine from blood and lymph following its intravenous administration. JAMES A. GREENE and GEORGE JOHNSTON (introduced by Fred M. Smith). The State University of Iowa, Iowa City.

The extremely rapid disappearance of tyrosine from the blood stream following intravenous administration has been observed by King and Rapport, and King, Simmonds and Aisner, but no attempt has been made to ascertain the rate of disappearance. The present investigation was undertaken to ascertain whether or not a tolerance curve could be obtained in normal dogs. The dogs were fasted for 18 to 24 hours and tyrosine was injected intravenously as described by King and Rapport. For the sake of uniformity 0.2 grams of tyrosine per kilo of body weight were given. Blood phenols were determined on 0.2 cc. blood samples utilizing the reagent of Folin and Ciocalteu, previous to and at intervals of from one minute to four hours following the injection. The results of 23 experiments on six normal dogs show a uniform disappearance rate.

This rate of disappearance was not altered by; acute chloroform poisoning, by a seven-day period of fasting, by a normal diet supplemented with seven daily intravenous injections of 0.2 gram of tyrosine per kilo of body weight, or by injection of 1 cc. of India ink per kilo body weight two hours previous to the test. The elevation of phenols in the thoracic duct lymph and their subsequent disappearance rate was found to be comparable with the blood following the intravenous injection of tyrosine as described above.

*Experimental intersexuality: Modifications of embryonic sexual development obtained by giving large amounts of estrogens to the pregnant rat.*¹ R. R. GREENE and M. W. BURRILL (introduced by A. C. Ivy). Department of Physiology, Northwestern University Medical School, Chicago, Ill. Large amounts of estrogens have been administered to pregnant rats.

In spite of the high dosages of estrogens used (highest dose: 100 mg. of estradiol dipropionate on the 13th day of pregnancy), 100 out of 212 injected animals have carried their pregnancies to term.

The male offspring are markedly feminized. Structures characteristic of the female have been stimulated to develop. Nipples, a vagina, and parts of the uteri are found. Structures characteristic of the male are inhibited. The phallus is hypospadiac, prostates are absent, seminal vesicles are very small. Portions, or almost all, of the vasa deferentia and epididymides may be absent. The gonads in well modified animals are intra-abdominal and are located at the base of the kidneys, a position which is typical of the female gonads.

¹ Supported in part by a grant from the Josiah Macy, Jr. Foundation.

The findings in the female offspring are rather inexplicable. There is stimulation of some female structures. Nipples are precociously present and the uteri are greatly enlarged. There is also inhibition of some female structures. Ovarian capsules are not present. Division of the urogenital sinus into vagina and urethra is partially inhibited. In the adult state the vagina and urethra open through a common orifice at the base of an hypospadiac phallus.

In these females the Wolffian duct is stimulated to persist and has been found to be partially or completely present in 15 serially sectioned new born females.

In the sense that development of certain female structures is inhibited and certain male structures are stimulated to persist, these females may be considered to have been masculinized.

However, the experimental procedure employed, i.e., the injection of estrogens into the pregnant rat, does not prove that the "masculinizing" effect on sexual development of the female embryo is a *direct* action of the estrogens.

Absorption and oxidation of orally administered colloidal sulphur. HARRY GREENGARD and JEAN REA WOLLEY (introduced by K. K. Jones). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

It is reported that when powdered sulphur is fed it is not absorbed, except insofar as it is converted to hydrogen sulphide by the intestinal flora; on the other hand, colloidal sulphur is stated to be rapidly absorbed and excreted in the urine, for the most part as unoxidized sulphur. We have noted that when a preparation of colloidal sulphur was given to rabbits in massive doses by stomach tube the toxic effects were manifested in a very few minutes, indicating an immediate absorption of sulphur. Furthermore, when a constant diet was supplemented with colloidal sulphur in rabbits and in human subjects there was an average daily excretion of extra sulphur exactly equal, within the limits of experimental error, to the amount administered; this extra sulphur was almost entirely in the form of sulphate. These findings indicate that the colloidal sulphur administered was promptly and completely absorbed, oxidized to sulphate, and excreted in the urine within twenty-four hours.

*Plasma volume and "available fluid" changes in dehydration.*¹ MAGNUS I. GREGERSEN and ELIZABETH PAINTER (by invitation). Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

Dehydration (8-19 per cent loss in body weight) was produced in dogs by water deprivation and by intravenous injection of hypertonic (50 per cent) sucrose. The changes in plasma volume, in fluid available for solution of sodium thiocyanate and (in some experiments) in the water available for solution of sulfanilamide were measured with methods described previously (Gregersen et al, 1935-39; Painter, 1938). In 14 experiments on water deprivation the average decrease in plasma volume was 26 per cent (17-41) and in the "available fluid" 17 per cent (8-28). Changes of similar magnitude were observed in animals dehydrated with

¹ Aided by a grant from the Committee on Grants-in-Aid, National Research Council.

sucrose diuresis. According to the results of experiments in which we attempted to determine total body water (sulfanilamide) as well as "available fluid", the fluid lost in dehydration is drawn from both the extra- and intracellular compartments.

The magnitude, adequacy and source of the collateral circulation in chronically occluded coronary arteries. DONALD E. GREGG, JOHN THORNTON (by invitation) and F. R. MAUTZ (by invitation). Departments of Physiology and Surgery, Western Reserve University, School of Medicine, Cleveland, O.

Following chronic arterial coronary occlusion in dogs lasting from one to twelve months, the magnitude, adequacy, and source of the collateral circulation established has been determined. In 22 of 23 dogs the peripheral coronary pressure is greatly increased (as compared to normal controls), the retrograde blood flow from the peripheral end of the occluded coronary is arterial (oxygen and carbon dioxide analyses), is generally greater in volume in the left coronary rami than in the right and ranges up to 105 cc. per minute with most of the values around 30-40 cc. per minute. Such volume flows of arterial blood are sufficient for the metabolic needs of the potentially infarcted region for the myocardium exhibits normal contractions except in occasional areas of scarring.

No single pulse pattern nor set of pressure values exists in any of the major coronary rami chronically occluded. The most usual curve is similar in timing and contour (although greater in magnitude) to the normal peripheral coronary pressure curve. Others more nearly resemble an intraventricular pressure curve while at times the curve is almost identical with the aortic pressure curve simultaneously recorded.

By clamping the other coronaries either separately or together while measuring the retrograde flow in the third coronary chronically occluded the extent of the intercoronary collateral flow has been established. The occluded descendens receives on an average 62-64 per cent of its collateral flow from the coronaries of which the right and circumflex contribute 7 and 55 per cent respectively. The chronic circumflex obtains 66-64 per cent of its flow from the other coronaries of which the right and descendens contribute 19 and 47 per cent while the chronic right receives 97-79 per cent of its flow from the descendens (22 per cent) and circumflex (75 per cent).

The variable relationship between differential pressure and blood flow in a coronary artery. DONALD E. GREGG and HAROLD D. GREEN. Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O. (Read by title.)

It has been postulated (Am. J. Physiol. **112**: 362, 627, 1935) that coronary blood flow is proportional to the moment to moment difference between aortic and peripheral coronary pressures. This hypothesis has now been tested by means of a constant pressure flow meter (demonstrated at this meeting). The flow of blood, infused at various pressures, has been compared with the differential pressure (difference between infusion and peripheral coronary pressure) at moments of maximum systolic and minimum diastolic coronary resistance.

Under essentially normal circulatory conditions it has been found that: 1, at infusion pressures equal either to systolic or diastolic coronary resist-

ances the flow stops momentarily during the corresponding period; 2, the diastolic flows bear an approximately linear relationship to the differential pressures in many determinations, but in others the flow varies as some power of the pressure; 3, a proportional relationship exists between differential pressures and flow during systole and diastole in some experiments but in others the systolic flow is 50 to 75 per cent of that calculated for diastole at the same differential pressure.

Under altered coronary dynamic conditions, such as temporary anemia of the vascular bed, increased cardiac output and substitution of Locke's solution for blood as the perfusate the flow meter has registered flow increases ranging from 50 to 300 per cent. Differential curves recorded at essentially the same time indicate the directional changes in systolic and diastolic flows but are less in magnitude than the actual blood flows measured by the meter.

It is concluded therefore that the peripheral coronary pressure curves indicate the exact time relations of the phasic changes of coronary flow and the proper values for systolic and diastolic resistances and that the differential pressure curves reveal the direction of change of phasic coronary flow, but cannot be used as an exact quantitative measure of the phasic flow.

A constant pressure flow meter. DONALD E. GREGG and HAROLD D. GREEN.
Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O. (Demonstration.)

Blood or other infusing fluid (5 to 10 cc.) is placed in a chamber which empties through a stopcock into a short piece of lead tubing (6 mm. internal bore) leading to the blood vessel. A side arm connects with a Gregg manometer fitted with a tight membrane (40 to 60 mm. deflection at 4 meters for 100 mm. Hg—average natural frequency 250+ per second), which records the pressure in the artery when fluid is not flowing (peripheral coronary pressure) and the infusion pressure during flow measurements. A short wide tube connects the airchamber with a Wiggers' type (ball and socket) manometer fitted with a sensitive membrane (200 mm. deflection at 4 meters for 10 mm. Hg—average natural frequency 150+ per second).

In operation the infusing fluid is placed in the meter after which the pressure in the air chamber is raised to the desired value and the flow beam (from the sensitive manometer) focused on the left side of the camera. During registration of flow the pressure in the air chamber drops slightly (usually less than 1 mm. per heart beat, the rate of drop of pressure depending upon the rate of outflow. These slight decreases of pressure are reflected in the flow beam by progressive movement to the right, the rate of movement reflecting the rate of flow.

The apparatus is calibrated by recording the movement of the flow beam at each infusion pressure during outflow of a measured volume of fluid. The time lag of the meter system is less than 0.005 sec. This was determined by forcing fluid into and out of the meter from a syringe by means of a reciprocating plunger while recording photographically the movement of the plunger and flow beam (1000 cycles/min., av. velocity flow 2000 cc./min.). Connecting the meter to a stopcock (for abruptly stopping and starting the flow) yielded records with sharp angular changes of flow without overshooting or appreciable lag.

The blood flow of the spleen. J. H. GRINDLAY (by invitation), J. F. HERICK and F. C. MANN. Division of Experimental Medicine, The Mayo Foundation and Mayo Clinic, Rochester, Minn.

Thermostromuhr units were placed on either the splenic artery or vein, using ether anesthesia and aseptic technic. This method permitted measurements of the blood flow of the spleen in the trained dog during basal and various experimental conditions. The blood flow of the splenic artery and vein were measured simultaneously in anesthetized dogs and the results obtained showed that arterial flow equalled venous flow within experimental error.

Hemorrhage, exercise, epinephrine, ephedrine, pitressin, histamine, acetylcholine, nitroglycerine, shivering due to cold, and a sudden loud noise were found to alter the blood flow in such manner that venous flow exceeded arterial flow for an interval. After this interval the blood flow in both vessels was less than for the control period for varying lengths of time in the case of hemorrhage, epinephrine, ephedrine, pitressin, and histamine. In the case of exercise and shivering the blood flow in the splenic vein and artery following the interval of relatively increased venous flow was larger than it was during the control period. Acetylcholine, nitroglycerine, and a sudden noise caused no further changes in blood flow.

Gradual and prolonged increases of the flow of blood in the splenic artery and vein were noted after the feeding of a standard meal to a fasted dog, after the intravenous injection of heparinized blood or hypertonic or isotonic solutions, or after sodium pentobarbital anesthesia.

This study has demonstrated that the flow of blood in the splenic vein exceeds that in the splenic artery for an interval during which the spleen is known to be contracting. The contracted spleen was found to have a decreased arterial and venous flow under certain circumstances, and an increased arterial and venous flow under other circumstances. In several conditions associated with enlargement of the spleen the blood flow likewise was increased.

Properties of mammalian preganglionic B fibers. H. GRUNDFEST. The Rockefeller Institute for Medical Research, New York City.

The fibers of mammalian preganglionic nerves which conduct with velocities of 15-3 m.p.s. (B fibers (B₂ Bishop and Heinbecker)) in single responses develop action potentials distinguished from A and C potentials in that they consist of a spike and positive after-potential without a visible intervening negative after-potential. B fibers asphyxiate more quickly than A and C fibers. Threshold spikes last 1.2 msec., as does absolute refractoriness in hypogastric nerves of the cat, and in cervical sympathetic nerves of the cat and rabbit. The spike height returns to normal after about 40 msec., but the excitability remains subnormal throughout the duration of the positive after-potential. Subliminal summation lasts 0.2 msec.

The positive after-potential becomes maximal at 20-30 msec., reaching then 1.5-4 per cent of the potential of the undispersed spike. At pH 7.4 the positivity lasts 100-300 msec. At pH 8.0 the duration characteristically shortens to 50-80 msec. Decreasing pH does not affect the positivity, but a negative after-potential may follow the spike. In veratrinized B fibers a large negative after-potential lasting 1-2 sec. obscures the original positivity.

In repetitive activity the positive after-potential developed by succeeding responses is smaller than that contributed by the first, and the base from which successive spikes arise may become negative. The potential sequence at the end is determined by the extent of activity. The terminal positivity is small after a short burst of activity and its duration may be curtailed. As the rates and durations of activity are increased, a second positive after-potential arises after the first has subsided. A short period of negativity may intervene. With more activity the second positivity develops earlier and becomes deeper and longer, until the two positive potentials fuse, reaching a maximum at 300 msec. and lasting over 1 sec.

The elements of the B action potential are, therefore, identical with those which appear in the action potentials of A and C fibers. In B fibers the negative after-potential is submerged, but it can manifest itself either overtly or through the production of a late positive after-potential.

The standardization of animals for glycogen determinations. M. MASON GUEST and RUTH A. RAWSON (introduced by E. L. Scott). Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

In glycogen determinations large deviations from animal to animal appear to be characteristic. In order to materially reduce this variability a standardization of controls has been attempted. The precautions taken and the results obtained are outlined below:

1. An inbred strain of Wistar rats is used.
2. Only males are used. (H. J. Deuel, L. F. Hallman, S. Murray, and L. T. Samuels. *J. Biol. Chem.* **119**: 607, 1937.)
3. The age of the animals is restricted to 100 ± 4 days at the time of the sampling. (H. J. Deuel, J. S. Butts, L. F. Hallman, S. Murray, and H. Blunden. *J. Biol. Chem.* **119**: 617, 1937.)
4. A uniform dry pellet diet which contains more than the minimum of all factors necessary for the maintenance of growth and health is fed.
5. An inanition period is established during the interval from 72 to 12 hours preceding the sampling. Food is given *ad lib.* during the final 12 hours, beginning at 10:00 p.m.
6. The liver and muscle samples are taken between 9:30 and 11:30 a.m. (H. J. Deuel, J. S. Butts, L. F. Hallman, S. Murray, and H. Blunden. *J. Biol. Chem.* **123**: 257, 1938.)
7. The weight of the dried stomach contents is subtracted from the weight of the food eaten and all animals in which less than 35 mgm. per gram of body weight has passed the stomach are excluded.
8. Since rats are nocturnally active a light is left on until 10:00 p.m., at the time the feeding begins.
9. The temperature of the environment is maintained at 28°C. during the 72 hours preceding the sampling.
10. Anesthesia is by intraperitoneal injection of 0.70 mgm. per kgm. body weight of nembutal. Induction occurs within 4 minutes.
11. The method of glycogen analysis is that described by N. R. Blatherwick, P. J. Bradshaw, M. E. Ewing, H. W. Larson and S. D. Sawyer (*J. Biol. Chem.* **111**: 537, 1935) with the addition of our previously mentioned precautions. (*J.B.C.* **123**: xlviii, 1938). Condensation on the ma-

terial has been further reduced by manipulation in a room maintained at 10°C.

The following results have been obtained when the outlined procedure was followed: Liver glycogen, $N = 50$; Mean = 6.9; $\epsilon = 0.9$ (13 per cent). Muscle glycogen, $N = 50$; Mean = 0.91; $\epsilon = 0.10$ (11 per cent).

The electrical impedance of muscle during the action of sugar, inorganic cations and narcotics. RITA GUTTMAN (introduced by K. S. Cole). College of Physicians and Surgeons, Columbia University, New York City.

The effects of sugar, inorganic cations and narcotics upon the impedance of frog sartorius muscle were studied by circulating isosmotic solutions past the muscle.

The penetration of sugar-Ringer solutions into the intercellular spaces, as studied by impedance changes, follows the diffusion law, the amount diffusing in being proportional to the square root of the time. Half of the impedance change is over in three quarters of a minute when the sugar solution is permitted to circulate past both sides of the muscle. This sets an upper limit for the time necessary for inorganic cations and organic narcotics to reach the cell surfaces. The action of inorganic cations and organic narcotics upon muscle is slow compared to the time necessary for them to reach the scene of action.

Average values of 78 per cent for the volume concentration of fibers, 230 Ohms specific resistance for the resistance of the interior of the fibers, and 71° for the phase angle of the impedance locus were found for the muscle in Ringer's solution. The effect of various concentrations of sugar upon these values was also studied.

The effect of the inorganic cations, Na, K, Ba, Ca and Mg upon 1000 cycle resistance of muscle was investigated. Na, K and Mg have little effect, while Ba and Ca cause a marked drop in resistance. The use of physiologically "unbalanced" salt solutions does not, in itself, seem to influence muscle impedance.

The action of a number of organic narcotics: saponin, chloroform, sodium taurocholate, butyl and amyl alcohol, iso amyl carbamate, chloral hydrate and sodium salicylate was studied. By investigating the effect of narcotics upon muscle impedance over a wide frequency range, it was found that during narcosis the membrane resistance of muscle fibers first increases and then decreases, and, if the drug is present in sufficiently great concentration, membrane resistance may completely disappear. Membrane capacity, on the other hand, remains relatively unaffected by narcotics.

Stabilization of nerve membranes by alkali earths, as manifested in resting potential measurements. RITA GUTTMAN (introduced by Kenneth S. Cole). Marine Biological Laboratory, Woods Hole, Mass., and College of Physicians and Surgeons, Columbia University, New York City. (Read by title.)

The alkali earths: Ba, Sr, Ca and Mg, in isosmotic solutions of their chlorides, have, in general, no effect upon the resting potential of the non-medullated spider crab nerve. Ba, Sr and Ca can, however, prevent the depressing action of K upon the resting potential. The order of effectiveness of these ions in this regard is the following: Ba, Sr, Ca.

Ba, Sr, Ca and Mg oppose the depressing action of veratrine upon the resting potential. The order of effectiveness is Ba, Sr, Ca, Mg. The relation between the drop in potential caused by veratrine and the logarithm of veratrine concentration is a linear one. The action of various other organic ions and molecules which depress the resting potential: saponin, iso amyl carbamate, chloral hydrate and sodium salicylate, can likewise be neutralized by the alkali earth ion barium.

Hypertonic sea water solutions do not affect the resting potential. Also, the nerves do not shrink in hypertonic solutions although they swell in hypotonic sea water.

The alkali earths depress excitability reversibly. The various organic agents which depress the resting potential also depress excitability, in most cases reversibly; however the quantities necessary for depressing excitability are much smaller than those necessary for depressing the resting potential.

The possible relation of these findings to the concept that the resting potential is largely a diffusion potential set up by a difference of K ion concentration inside and outside the nerve fiber is considered. Whether the findings outlined above hold for the sciatic nerve and the sartorius muscle of the frog is in the process of being investigated.

Chemical pacemakers for alpha brain wave frequencies in general paresis.

ZAREH HADIDIAN (by invitation) and HUDSON HOAGLAND. The Biological Laboratories, Clark University, Worcester, Mass.

Alpha frequencies are modified by metabolic stimulants and inhibitors indicating that they (as rates) measure cortical respiration. Hoagland (Am. J. Physiol. **116**: 604, 1936) has shown that the frequency of human alpha brain waves is described by the Arrhenius equation as a function of temperature, yielding energies of activation (μ values) of ca. 8000 calories for normals and early general paretics, ca. 11,000 calories for intermediately advanced paretics, and ca. 16,000 for advanced paretics. These values agree with those frequently encountered in cellular respiration in vitro and it was suggested that they correspond to energies of activation of specific enzyme systems involved in cellular respiration in which the slowest link might be the "pacemaker" determining the overall μ value if frequency were proportional to respiratory rate.

It is known that the oxidation of Fe^{++} to Fe^{+++} gives μ of ca. 16,000 calories, making the indophenoloxidase-cytochrome system suspect as pacemaker where this value so frequently occurs in respiration. As a result of syphilitic brain infection *some* endogenous brain iron is evidently precipitated out, (Hoagland, in press) a condition which might tend to slow iron catalysed steps in respiration and thus bring about a μ of 16,000 in advanced paretics.

If this view is correct, it should be possible to obtain a respiratory enzyme and substrate system, measure its μ value for O_2 uptake and then shift the μ to 16,000 calories by adding cyanide to poison *some* of the indophenoloxidase-cytochrome and make it the slow link. Stotz and Hastings (J. Biol. Chem. **118**: 479, 1937) made a beef heart extract containing only two enzymes, indophenoloxidase-cytochrome and succino-dehydrogenase. This preparation oxidizes succinate to fumarate. The succino-dehydrogenase is not CN sensitive. Fumarase is not present. This system we find yields a μ value of 11,200 calories. Addition of 0.0001

mM NaCn, while decreasing O_2 consumption, does not shift the μ . Addition of 0.0015 mM NaCn or more shifts the μ value cleanly to 16,000 calories. All O_2 consumption stops with 0.006 mM NaCn. Evidently 16,000 calories is the energy of activation of cytochrome and its oxidase and 11,200 is that for succino-dehydrogenase.

Dark adaptation measurements with natural and constant pupillary apertures.

CHARLES HAIG (introduced by Selig Hecht). Research Division of the Welfare Hospital for Chronic Disease, and the Department of Medicine, Columbia University, New York City.

Measurements of the rod dark adaptation of two subjects made with a constant pupillary aperture and with the natural pupil, following various intensities of light adaptation, show differences in form which are greater the lower the pre-adapting intensity. These differences are caused by the effect on the size of the pupil of the fixation light used in the observations. The result is that the pupil, after first dilating, later becomes smaller as dark adaptation proceeds, and finally dilates again toward the end of the process. It is concluded that for precise measurements of dark adaptation an artificial or fixed pupil must be employed.

Since in measurements following adaptation for more than 3 minutes to intensities greater than 1000 millilamberts the error produced in this manner by the pupillary light reflex is smaller than the day to day variations, it is deemed unnecessary to employ an artificial pupil in clinical measurements made under these conditions.

Measurements made with a constant pupillary aperture are found to have the same mathematical form independently of the degree of light adaptation. Moreover, for a given degree of light adaptation, whether produced by brief exposure to a bright light or prolonged exposure to one of low intensity, the course of dark adaptation is the same. It is concluded that variations in the rate of dark adaptation accompanying changes in the degree of light adaptation are not mediated by a cyclic mechanism involving a fast and a slow chemical process.

Diet and the insulin content of pancreas. REGINALD E. HAIST (by invitation), JESSIE H. RIDOUT (by invitation) and C. H. BEST. Department of Physiology and the School of Hygiene, University of Toronto, Toronto, Canada.

In order to investigate the effect of dietary changes on the insulin content of pancreas it is necessary to have available, first, a method which consistently gives a maximal yield of anti-diabetic substance; secondly, a method of testing insulin which gives accurate results with the amount of active material available; and thirdly, a test animal which will consistently eat the diets provided and whose pancreas can be completely removed without undue difficulty. With regard to the first requirement the method recently described by Scott and Fisher,¹ with very slight modifications, is satisfactory. The mouse method of assay, using from 200 to 300 mice for each sample of insulin-containing extract, has been found to give remarkably consistent results. White rats of the Wistar strain are suitable test animals. All tests were made on the pancreatic tissue from 10 rats which yields, in normal animals, from 20 to 30 units of insulin.

¹ Scott, D. A. and A. M. Fisher. Am. J. Physiol. **121**: 253, 1938.

Utilizing these procedures, we have studied the changes in insulin content of rat pancreas under a variety of dietary conditions. It has been found that starvation produces a very definite decrease in the insulin content of pancreas. Diets very rich in fat cause a marked diminution in insulin content. Diets rich in carbohydrate tend to prevent the fall in insulin content. The insulin content, reduced by a 7-day fast, may be increased by feeding a balanced diet or sugar only, but not by feeding fat. Neither diets deficient in vitamin B₁ nor the daily administration of large amounts of adrenal cortical extract gave results which were significantly different from those of the paired-fed controls.

The effects on the Ca and P balance and on various bodily constituents of the rat produced by an increase in the Ca and P content of the diet. JOHN HALDI, GEORGE BACHMAN, WINFREY WYNN (by invitation) and CHARLES ENSOR (by invitation). Laboratory of Physiology, Emory University School of Medicine, Emory University, Ga.

Fourteen groups of albino rats with three litter mates in each group were placed on the Wistar diet at weaning and one week later on a basal diet containing 68 per cent sucrose. The three rations contained the Osborne-Mendel salt mixture. To rations 2 and 3 was added a neutral combination of NaH₂PO₄ and Na₂HPO₄ in such amounts that they contained 2.5 and 3.2 times as much phosphorus as in ration 1. The Ca/P ratio was maintained within the range 1.5-1.9 by the addition of calcium carbonate. Litter mates consumed equicaloric amounts of food. They were kept on the rations for 70 days.

An increase in Ca intake from 7 grams (controls) to 14 and 17.8 grams respectively led to an increased absorption of only 0.3 and 0.9 gram and an increased deposition in the body of 0.04 and 0.15 gram. The excess of absorbed over stored Ca was presumably excreted in the urine.

With an increase in the P intake from 3.7 to 9.1 and 11.8 grams the amount absorbed was increased 3.5 and 4.7 grams respectively. The amount deposited in the body in both instances was the same as in the controls. A large amount of phosphorus was therefore eliminated by way of the kidneys.

The water intake on the three rations was 1168, 1642 and 2531 cc. respectively on the three rations.

With an increase in Ca and P intake there was a progressive diminution in the fat content of the body from 28.1 to 23.1 and 18.0 grams. There was a progressive increase in the combustible material of the feces which suggests an impairment of digestibility or of absorption. This in turn might account at least in part for the diminution in fat deposition. Paralleling the lower fat content of the body there was an increase in the water content from 59.2 to 61.2 and 63.3 per cent. The dry weight of the body on the three rations was 75.8, 70.9 and 64.6 grams.

The unfavorable effects of the experimental rations are attributed to the phosphorus since there was only a small increase in the calcium absorption.

Influence of light on the response of the capon comb to directly applied androgen. SAM R. HALL and L. P. DRYDEN (introduced by Maurice H. Friedman). Bureau of Dairy Industry, U. S. Department of Agriculture. (Read by title.)

Womack and Koch (Proc. 2nd Inter. Cong. Sex Res., 1930) found that capons injected with androgen and kept in the dark over a period of ten

days showed a much greater comb response than a control group similarly injected and exposed to outside light conditions. They do not mention the season of the year in which their experiment was performed.

During late October, 35 eight-month-old capons all of the same strain, caponized at four weeks of age and which had been used only once (three weeks earlier) were divided into three approximately equal groups. The birds were so selected that the three groups should give equal average responses as judged by the single previous use. They all received directly to the surface of the comb the same solution of 0.5 gamma androsterone in $\frac{1}{8}$ cc. tricaproin once daily for the duration of the experiment. Length plus height in millimeters was used as the index of comb size.

The table summarizes the results under varying light conditions. Groups B and C of October were reversed for the December confirmatory experiment whereas group A remained the same.

| GROUP | LIGHT CONDITIONS | NUMBER OF BIRDS | DATE | 5 DAYS | | | 10 DAYS | | |
|-------|------------------|-----------------|---------|--------|-----------|------------|---------|-----------|------------|
| | | | | A.M. | S.D. | S.E. | A.M. | S.D. | S.E. |
| A | Normal light | 16 | Oct. 3 | 4.7 | ± 1.6 | ± 0.41 | | | |
| | Normal light | 12 | Oct. 31 | 4.7 | ± 1.8 | ± 0.53 | | | |
| A | Normal light | 11 | Dec. 5 | 4.5 | ± 1.7 | ± 0.50 | 7.3 | ± 2.4 | ± 0.72 |
| B | Constant dark | 11 | Oct. 31 | 5.0 | ± 2.5 | ± 0.75 | | | |
| C | Constant dark | 11 | Dec. 5 | 4.5 | ± 2.3 | ± 0.71 | 7.3 | ± 3.1 | ± 0.93 |
| C | Constant light | 12 | Oct. 31 | 3.5 | ± 1.4 | ± 0.42 | | | |
| B | Constant light | 9 | Dec. 5 | 3.0 | ± 1.6 | ± 0.53 | 4.8 | ± 1.9 | ± 0.64 |

From the table it is seen that at this dose level and at this time of the year the response elicited by the direct application of this androgen to the capon's comb is not increased over normal day and night conditions by constant darkness. Constant artificial light does, however, especially after ten days, decrease the response as compared to either constant darkness or normal lighting conditions.

Influence of season and other factors on the response of the capon comb to directly applied androsterone. SAM R. HALL and JOHN D. HUNT (introduced by Maurice H. Friedman). Bureau of Dairy Industry, U. S. Department of Agriculture. (Read by title.)

K. David (*Acta Brevia Neerland* 8: 1938) concluded from injection experiments with capons that there was a 100 per cent greater sensitivity in the comb in the winter months than in the summer. We find by the inunction method in 76 measurements in one group from early summer to winter no increase in responsiveness and in 86 tests in another group from early fall to late winter likewise no increase. Our data actually indicate a lessened response in the winter.

These experiments were conducted on capons obtained from two different sources. Between these two strains of White Leghorn capons, there is a slight but significant difference in sensitivity. A dose of 0.5 gamma in $\frac{1}{8}$ cc. oil in 45 measurements in strain I which had been caponized at five weeks of age gave an average of 5.11 mm. increase with a standard error of $=0.257$; one hundred and two measurements in strain II caponized at four weeks of age gave 4.36 with a S.E. of $=0.128$. This comparison was made during identical months.

Sixteen capons six months old that had never been used before gave at 0.5 gamma 4.7 mm. increase on October 3. Twelve of the same strain three weeks later on their second usage gave 4.7. The third time, four weeks later, eleven gave 4.5; the fourth time, four weeks later, twenty-eight from this whole group gave 4.3; and the fifth time, four weeks later, nineteen gave 4.4. The last measurements were made on February 11. These data also illustrate the lack of increased sensitivity due to the approach of winter, advancing age, and/or accumulated usage.

Intervals of three to eight weeks between uses have not changed the response. Neither has the magnitude of the previous response, at the level studied, changed the subsequent response significantly.

We find no correlation between initial comb size and average or individual response within the limits studied. "Slips" were not included in the initial comb size data; neither were they used in any of our averages. They are easily recognized and have larger combs which are usually more sensitive.

Change of hair from gray to a darker color following administration of male hormone substance to castrated men. JAMES B. HAMILTON and GILBERT R. HUBERT (by invitation). Yale University School of Medicine, New Haven, Conn., and Albany Hospital, Albany, N. Y. (Read by title.)

The skin of castrated and eunuchoid men has been observed to lose its pasty sallow color and to become pigmented and flushed during the administration of testosterone propionate (Science **88**: 481, 1938). Similar phenomena have been found in ovariectomized women who have received either estrogenic or androgenic treatment. Recently changes in hair coloration have been seen to accompany those in the skin. In 3 of 4 castrated men between 35 and 45 years of age, who had been injected with 20 mgm. of testosterone propionate daily for several months, the hair of the head, especially about the region of the ears, assumed a darker color. After cessation of treatment, the hair, like the skin, again appeared lighter in color. The limited data obtained thus far regarding the physiological and histological factors involved make unwise further interpretation and the extension of these phenomena to other conditions than those stated.

Studies in iodine metabolism by the use of a new radio-active isotope of iodine.

JOSEPH G. HAMILTON and MAYO H. SOLEY (introduced by C. D. Leake).

Radiation Laboratory, University of California, Berkeley, Division of Medicine and the Pharmacological Laboratory, University of California Medical School, San Francisco.

A recently discovered radio-active isotope of iodine with a half-life of eight days was used in a study of iodine metabolism in: 1, a group of patients with goiters of various degrees of functional activity; 2, two subjects with spontaneous myxedema; and 3, a group of normal subjects. The radio-active iodine was administered as a single dose in the form of a solution of sodium iodide which contained 14 mgm. of iodine. Twenty-four hour urine samples were collected for five successive days; the feces were collected during this five-day period and studied as one specimen; and the thyroid glands of the patients who came to surgery were removed two days after the radio-active iodine had been given. The radio-active iodine content of all these specimens was determined quantitatively, and

the total iodine content of most of the thyroid glands was measured quantitatively.

Fifty-three to 82 per cent of the iodine was excreted in the urine in 48 hours, and 12.3 to 15.4 per cent in the succeeding 72 hours. In the five days' samples of feces 0.08 to 16.7 per cent was excreted. The thyroid glands contained from 0.072 to 17.5 per cent of the radio-active iodine.

Pressure pulses in a case of aortic coarctation. W. F. HAMILTON, EUGENE E. MURPHEY (by invitation) and R. A. WOODBURY. Department of Physiology and Pharmacology and the Department of Medicine, University of Georgia School of Medicine, Augusta.

Through the cooperation of the departments of Medicine and Surgery of the University Hospital a case of coarctation of the aorta was made available for study. The marked activity of the great vessels in the neck the definite precordial heaving and forcible apex beat, together with the fact that there was no visible evidence of pulsation in the abdominal aorta or femoral arteries led to the conclusion that the aortic Windkessel was divided into two segments, one above and one below an obstruction. There was evidently a sufficient collateral circulation to maintain the lower parts of the body in good muscular development, but this collateral circulation was at the same time sluggish enough to make possible the separate study of the two reservoirs and their branches.

Direct optical blood pressure tracings were made simultaneously from various arteries. Pressure within arteries above the coarctation were 160/88 mm. Hg (mean 113); below the stricture they were 105/82 mm. Hg (mean 93). Pressure pulse contours in the upper system were normal; in the lower system they were flat, smooth, and somewhat delayed.

Coughing and straining produced a simultaneous rise in both systems. This pressure increase occurred separately in the two systems, i.e., it was not transmitted across the collateral circulation as was the pulse wave.

After intravenous epinephrine the pressure rose in the upper arteries, fell in the lower arteries; later it rose in both systems. The records indicate that there was a constriction of the collateral circulation and of the peripheral arterioles as well as an increase in the elasticity of the larger vessels.

Amyl nitrite produced changes of a nearly opposite description. Pressure in the upper system decreased; in the lower system it increased; and the transmission time between the systems decreased. The records indicate that the patient reacted against the vasomotor action of the drug by increased cardiac output and, during recovery, this factor increased the arterial pressure in the upper system to an unusually high value (234/123 mm. Hg).

The effect of bile on the secretion of the small intestine. H. F. HANEY and W. C. ROLEY (introduced by W. J. Meek). University of Oregon Medical School, Portland.

Thiry loops were prepared from the upper jejunum of healthy adult dogs. Each animal was trained to lie quietly throughout the period of a test. A fast of approximately 24 hours preceded each experiment. At the beginning of a test the loop was thoroughly washed out with physiologi-

cal saline and all contents aspirated. A 4 cc. portion of the solution to be tested was then introduced through a small rubber catheter. The fluid draining from the loop was collected during a 10 minute period at the end of which 4 cc. of the test solution were again injected and the collection continued as before. Each experiment consisted of 6 injections of the test fluid. At the end of the hour the loop was again washed with physiological saline and the washings pooled with the collection from the loop. Each experiment consisted of a control period of 1 hour during which physiological saline buffered to a pH of 6.4 to 6.8 was used as the test solution and a period of equal duration during which either dog's gall bladder bile or a buffered isotonic sodium taurocholate was used. One-fourth of the volume of fluid collected during a given hour test was incubated at 37°C. for 24 hours after adding $\frac{1}{2}$ gram of sucrose and $\frac{1}{2}$ cc. of toluene and diluting to 50 cc. The amount of reducing sugar present at the end of the incubation period was determined by the Shafer-Somogyi method on protein-free filtrates. A typical experiment resulted in a loss of 2 cc. from the control solution and a gain of 8 cc. in a taurocholate solution. In the same experiment the amount of reducing substance after incubation with sucrose was 277 mgm. per cent in the control and 629 mgm. per cent in the one in which bile salt was used. An additional control series in which the buffered physiological saline was used during two consecutive one-hour periods failed to show any increase of secretion during the second hour. In all but one of twenty experiments on two dogs thus far completed bile or bile salt solutions have produced significant increases in both the volume of secretion and the inverting enzyme production.

Studies on pain sensation. 1. *Measurement of pain threshold with thermal radiation.* J. D. HARDY (by invitation), H. G. WOLFF and H. GOODELL (by invitation). Russell Sage Institute of Pathology in affiliation with the New York Hospital, and Department of Medicine, Cornell University Medical College, New York City.

Using the following technique the threshold for pain has been studied in three normal individuals (two men and one woman, each weighing 65 kgm.). The light from a 1000 W bulb was focussed upon the blackened foreheads of the subjects. A shutter arrangement was provided to allow exposure to the radiation for exactly three seconds. The intensity of the radiation was varied by means of a rheostat until the subject just felt pain at the end of the exposure. This intensity was then measured radiometrically in gm. cals./sec./cm.² and was defined as the pain threshold. The results of the studies are:

1. The threshold for pain on the forehead was measured over a period of four months and was observed to be constant and independent of the emotional and physical state of the subject. The intensity of radiation required to evoke a painful sensation in three seconds was 0.218 ± 0.008 gm. cals./sec./cm.²

2. The intensity of the stimulus required to produce pain was the same regardless of the size of the skin area stimulated.

3. The stimulus was found to be independent of area even though the threshold had been raised by means of a drug. This evidence strongly suggests that there is no spatial summation of pain sensation such as has been found with temperature sense and vision.

4. Acetylsalicylic acid was observed to raise the threshold to pain but not to heat sense, thus demonstrating the independence of the two modalities.

5. The time required to evoke pain sensation was a function of the reciprocal of the intensity of the stimulus. No pain was evoked for radiation intensities smaller than 0.06 gm. cal./sec./cm.² even after several minutes of exposure.

6. Pain produced by this method was not dependent entirely upon how high the skin temperature was raised as pain could be evoked readily at temperatures less than 37°C.

The influence of frequency of hypothalamic stimulation upon the response.

KENDRICK HARE and WILLIAM A. GEOHEGAN (by invitation). Department of Physiology, Cornell University Medical College, New York City.

In twelve cats under ether, chloralose, or nembutal anesthesia, the hypothalamus was stimulated with bipolar electrodes manipulated with a Horsley-Clarke apparatus. Blood pressure was recorded with a mercury manometer connected to the femoral artery, respiration with a tambour and an intrapleural cannula. Observations were made upon somatic movements and pupillary responses. The hypothalamus was explored until an area responsive to electrical excitation was discovered. The electrodes were fixed in position, and the same area stimulated for a number of ten-second periods with condenser discharges of different frequencies. During each experiment, oscillographic analysis established that the voltage and configuration of the stimuli were not affected by frequency changes of from 2 to 1600 per second.

In most cases it was found that the magnitude of the response increased with frequencies up to several hundred per second. Beyond this optimum, increases in frequency produced a progressive diminution of response. Neither facilitation nor fatigue were responsible for the changes in response, for the entire series of records could be repeated, immediately or after a rest of half an hour, and each frequency would elicit its characteristic response. Differences of spread of current at different frequencies do not account for the change in response. In some instances, changes in frequency altered the character of the response as well as the magnitude. For example, low frequency of stimulation produced a fall in blood pressure, constriction of the pupils, a slowing of respiration; whereas high frequency stimulation of the same area caused a rise in blood pressure, dilatation of the pupils, and an increase in the rate and depth of respiration. After-discharge and postural reactions were also affected by frequency.

The life duration of the red blood cell (rat). O. G. HARNE, JOHN F. LUTZ (by invitation) and CARL L. DAVIS (by invitation). Department of Histology, University of Maryland School of Medicine, Baltimore.

Reticuloocytes supravitaly stained with brilliant cresyl blue were used as the index for blood replacement following hemorrhage, and the mass destruction of erythrocytes within the body. By the use of this principle a total of twenty-two rats were studied. Control reticulocyte levels were made over periods up to sixty-five days and were found to average 1.8 per cent. Animals were then depleted of blood in amounts varying from

0.17 to 2.5 per cent of their body weights. Reticulocyte counts were continued daily thereafter and resulting values plotted.

Two types of initial response to hemorrhage were noted. A "primary reticulocytosis" which rose to a peak in from two to four days, or a "secondary reticulocytosis" requiring nine days. These initial responses are followed at intervals of eight or nine days by spontaneous reticulocyte showers which continue until erythrocyte replacement is complete.

Seventy-three per cent of the animals showed the "primary reticulocytosis" with subsequent spontaneous showers. Nine per cent gave the "secondary" response, which is always weak and is commonly followed by a single spontaneous shower. Eighteen per cent responded in a complicated manner evincing a composite reaction.

In every case hemorrhage equivalent to 0.17 per cent or more of the body weight produced a reticulocytosis.

The simplest curve recorded is that of the primary reticulocytosis. This initial peak varies in extent with the degree of hemorrhage. It is followed at intervals of eight or nine days by spontaneous reticulocyte showers which we have interpreted as resulting from the mass senescence and disappearance of an entire generation of erythrocytes once used for replacement. The frequency of this cycle which in the rat is eight or nine days thus represents the life duration of the red blood cell.

Using these data, the "secondary reticulocytosis" with its weaker response, and the complicated "composite" reaction found in a few animals may also be interpreted accordingly.

*Cardio-inhibitory and vaso-depressor reflexes from the nose and throat.*¹

A. SIDNEY HARRIS (introduced by Joseph Erlanger). Department of Otolaryngology, Washington University School of Medicine, St. Louis, Mo.

The heart can be stopped momentarily or greatly slowed for many seconds, and the blood pressure lowered by stimuli applied to the mucosa of the respiratory tract. These facts are well established by old experimental observations which also showed the cardiac slowing to be due to a bulbar reflex with the vagus nerve the centrifugal pathway.

In some preliminary experiments with rabbits, tracheotomized about three weeks before, blowing a suitable quantity of irritant vapor through the pharynx and nasal cavity resulted in slowing of the heart to about 20 per cent of the normal rate. The period of great slowing lasted about forty seconds and was accompanied by suspension of respiration. Animals with nasal windows for the application of stimuli to the mucosa may be kept in good health and used for an indefinite length of time in experiments not requiring further surgery. Individual animals differ greatly in sensitivity, but the same animal exhibits a high degree of constancy.

Mercury manometer records showing the changes in both heart rate and mean arterial pressure permit some analysis in terms of the mechanisms involved. The various reflex patterns evoked by chemical or mechanical stimuli to the mucosa, or electrical stimuli to the nerves supplying the mucosa, are as follows: 1, fall in arterial pressure with little change in rate; 2, fall in pressure accompanied or preceded by a reduction

¹ Aided by a grant from Philip Morris and Company, Ltd.

of rate; 3, great slowing of the heart with little or no change in pressure, usually followed by a slow rise; 4, rise in arterial pressure followed and accompanied by cardiac slowing (carotid sinus effect), and 5, rise in arterial pressure with little or no change in rate (under anesthesia).

In the animals whose primary depressor mechanisms are most exquisitely sensitive, patterns 1, 2 and 3 predominate, but the induction of anesthesia can cause the demonstration of 4 and 5. With all strong stimuli, respiration is inhibited. The experimental separability of *a*, vaso-depression; *b*, cardiac-inhibition, and *c*, increased arterial pressure with carotid sinus cardio-inhibition indicates that each of these response patterns has its own neural mechanism.

*The effect of anterior pituitary extract on the metabolism of fasting normal and adrenalectomised rats.*¹ HELEN C. HARRISON (by invitation) and C. N. H. LONG. Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven, Conn. (Read by title.)

The injection of crude anterior pituitary extracts into *fed* animals is followed *a*, by hyperglycaemia and glycosuria; *b*, by a reduction in urinary nitrogen and blood N.P.N. The latter effect has been attributed to the presence in these extracts of a "growth promoting" principle, but our experiments suggest that if these effects on protein metabolism are due to the "growth" hormone then it would be more advisable to regard this hormone as one endowed with the ability either to increase protein synthesis in fed animals or to retard its catabolism in fasted animals.

| TREATMENT | NUMBER OF RATS | URINE NITROGEN, MG./100 G/DAY | | | URINE KETONES, MG./100 G/DAY | | | TERMINAL BLOOD | |
|---|----------------|-------------------------------|--------|--------|------------------------------|--------|--------|----------------|--------|
| | | 1 day | 2 days | 3 days | 1 day | 2 days | 3 days | Glucose | NPN |
| | | | | | | | | mgm. % | mgm. % |
| Normal rats, fasted 72 hours. | 28 | 91 ± 2 | 78 ± 3 | 81 ± 4 | 0.4 | 2.1 | 2.2 | 82 ± 2 | 40 ± 2 |
| Normal rats, fasted 72 hours. A.P.E. 2nd and 3rd day. | 15 | | 65 ± 3 | 58 ± 2 | 0.5 | 17.7 | 12.6 | 57 ± 2 | 29 ± 1 |
| Adrenalectomised rats, fasted 72 hours. | 11 | 83 ± 4 | 66 ± 4 | 50 ± 2 | 0.4 | 0.7 | | 50 ± 3 | 41 ± 1 |
| Adrenalectomised rats, fasted 48 hours. A.P.E. 1st and 2nd day. | 8 | 61 ± 5 | 47 ± 4 | | 1.2 | 15.0 | | 27 ± 5 | 29 ± 4 |

This suggestion is based on experiments outlined in the table. It will be observed that the injection of crude saline extract of anterior pituitary (1 cc. three times a day) into both normal and adrenalectomised fasted rats is followed by *hypoglycemia*, *acetonuria*, and a reduction in urinary nitrogen and blood N.P.N. The association of these effects may be due to the separate action of different hormones but it seems equally probable that an approximately 30 per cent reduction in protein catabolism would not only increase the proportion of fat utilized, hence inducing *acetonuria*, but would also induce *hypoglycaemia* by reducing the quantity of material available for glucose formation.

¹ This work was assisted by grants from the Committee on Research in Endocrinology, National Research Council and The Fluid Research Fund, Yale University School of Medicine.

It may also be pointed out that fasted adrenalectomised rats maintained in good condition by the daily injection of hypertonic CaCl NaHCO_3 solution develop hypoglycaemia and that this is accompanied by a reduction in protein catabolism.

These results taken in conjunction with the work of Katzin and Long (cf. these Proceedings), on the effect of cortical extract on the protein metabolism of fasted rats, suggests that the anterior pituitary exerts through the adrenotropic hormone a stimulating, and through the "growth" hormone a depressing influence on protein catabolism in fasting animals.

Excitation and inhibition of the "off" response in vertebrate optic nerve fibers. H. K. HARTLINE. Johnson Foundation, University of Pennsylvania, Philadelphia.

In the vertebrate optic nerve nearly one third of the fibers respond only to cessation, or sudden reduction in intensity, of retinal illumination. The responses from a given fiber of this kind are greater the more intense the illumination and the more complete its reduction in intensity. They are stronger the greater the retinal area illuminated (within the limits of the receptive field of the fiber). Responses are obtained to decreasing the area, but not to increasing it. The effectiveness of a small spot of light depends on its location within the receptive field of the fiber, the sensitivity of which is greatest at its center. A sudden movement of a spot of light produces a response, but only if the movement carries it to a less sensitive region of the receptive field. These observations indicate that "off" responses are dependent upon retinal illumination for their excitation, and that the effects of excitation from different regions of the receptive field converge upon the ganglion cell, and are summed in the total excitation produced. Any procedure which abruptly lowers the amount of excitation produced by light is accompanied by an "off" response, in a fiber of this kind.

"Off" responses are abruptly cut short by re-illuminating either the same region of the retina used to elicit them, or an adjacent region within the receptive field of the fiber. This suppression is complete if the intensity of re-illumination equals or exceeds that used to elicit the response, and if a region of the receptive field of equal or greater sensitivity is re-illuminated. The greater the area re-illuminated the more effective is the suppression of the discharge. Thus there are inhibitory effects due to illumination, which converge upon the ganglion cell from all portions of its receptive field, and are summed in the total inhibition produced. This inhibitory process has its maximal effectiveness shortly after the onset of re-illumination (latency of 0.1 sec.), and subsides in several seconds to a lower level which remains greater than zero throughout illumination.

*The sodium factor of the adrenal.*¹ FRANK A. HARTMAN, HERBERT J. SPOOR (by invitation), LENA A. LEWIS (by invitation) and JANE E. GABRIEL (by invitation). Department of Physiology, The Ohio State University, Columbus.

It has been found possible to separate adrenal extract into two fractions, one containing the vital factor, cortin, which produces little or no sodium

¹ Aided by a grant from The Rockefeller Foundation.

retention and the other containing a factor responsible for sodium retention which we propose to call the sodium factor. The latter is low in vital potency as assayed on adrenalectomized cats. The former contained little or no sodium factor as assayed by determining its effect on the sodium retention of normal dogs.

The response of five adrenalectomized cats and two adrenalectomized dogs to the two factors has been studied. Cortin alone maintains these animals in good condition but the plasma sodium remains at the level, characteristic of adrenal insufficiency. Addition of the sodium factor promptly raises the plasma sodium to normal. Adrenalectomized animals treated with cortin alone apparently maintain a normal blood pressure.

There is no evidence that the sodium factor is corticosterone or desoxycorticosterone since either of these crystalline materials is effective in causing sodium retention after an animal has been made refractory to beef sodium factor. Moreover it has not been possible to make an animal refractory to corticosterone yet this is a characteristic property of the sodium factor.

On the mechanism of inhibition of water diuresis by certain afferent stimuli.

HANS O. HATERIUS. Department of Physiology, Wayne University College of Medicine, Detroit, Mich.

Since suitable afferent nerve stimuli will inhibit water diuresis in the dog, it has been suggested (Verney) that such stimuli may act reflexly upon the pituitary to bring about the curtailment of urine flow through liberation of antidiuretic hormone. An alternative possibility, an effect through nervous channels acting directly to cause tissue retention of fluid or to effect vasomotor redistribution of blood, has also been mentioned. Direct evidence one way or the other, however, has been lacking.

Adult rabbits, placed on a lettuce and water ration the day before, were prepared by a "priming" dose of water *per os*, 4 per cent of body weight. Following a second similar dose of water, under chloralose-urethane anaesthesia, and when the diuresis curve was rising rapidly, a needle was inserted into the lumbar region and moved vigorously about for ten minutes. Urine flow fell off sharply, in some cases nearly to basal level from a diuretic rate of 25-45 cc./ $\frac{1}{2}$ hr., and remained low for periods ranging to 2 $\frac{1}{2}$ hours, after which it slowly rose again. Failure of the secondary rise to occur was sufficient cause for rejection of the experiment. Following this confirmation, in the rabbit, of Theobald's observations in the dog, the pituitary stalks in a series of animals were destroyed by electrolytic lesion (method of Haterius and Ferguson, *Am. J. Physiol.* **124**: 314, 1938)—an operative procedure which was carefully verified at autopsy. Two to four days later the animals were prepared for water diuresis, as before. Stimulation of the lumbar region, even when prolonged to twenty minutes and made very drastic, failed—beyond an occasional slight depression during stimulation—to produce an appreciable lowering in the rate of urine flow; typically, it kept rising to a high peak despite the stimuli.

It appears, therefore, that interruption of the pituitary stalk is effective in blocking the antidiuretic response to afferent stimuli of the type employed, a circumstance which would seem to provide conclusive substantiation of Verney's pituitary hypothesis.

Diffusion of calcium, magnesium and phosphorus into the peritoneum. The effect of intramuscular injection of magnesium. VICTOR G. HAURY (by invitation) and A. CANTAROW. Departments of Pharmacology and Medicine, Jefferson Medical College and the Laboratory of Biochemistry, Jefferson Hospital, Philadelphia, Pa. (Read by title.)

Four dogs were given dextrose (2.5 per cent) and saline (0.9 per cent) solution intraperitoneally (100 cc. per kgm.). After 16 hours blood (femoral artery) and peritoneal fluid (20 cc. each) were withdrawn and 50 cc. of a 25 per cent solution of magnesium sulphate were injected intramuscularly. Blood and fluid samples were obtained $\frac{1}{2}$, 1, 2, 3 and 4 $\frac{1}{2}$ hours after injection.

The serum Mg rose from control levels of 1.82-2.46 mgm. to a maximum of 8.9-16.1 mgm. in $\frac{1}{2}$ to 3 hours, with a subsequent decline. The peritoneal fluid Mg rose from control levels of 1.5-1.67 mgm. to a maximum of 6.4-12.2 mgm., the peak being reached 1 $\frac{1}{2}$ hours after the peak of serum Mg concentration.

The serum Ca fell from control levels of 9.45-12.1 mgm. to minimum concentrations of 6.39, 7.0, 7.6 and 8.92 mgm., these values being reached 1-1 $\frac{1}{2}$ hours after the peak of serum Mg concentration. The control values for peritoneal fluid Ca concentration were 5.94-8.5 mgm. This remained essentially unchanged except in one case in which it fell from 6.7 mgm. to 4.6 mgm. at the end of 3 hours. Frank tetany occurred in the three animals in which the serum Ca concentration fell below 8 mgm. per cent despite the absence of significant alteration in peritoneal fluid Ca in two of these animals and the presence of high Mg concentrations in both serum and peritoneal fluid.

A significant fall occurred in serum and peritoneal fluid inorganic P in 2 of the 3 cases in which this determination was made, the minimum values being obtained 1 and 1 $\frac{1}{2}$ hours respectively after the serum Mg had attained a maximum concentration.

Intra-individual differences in oxygen consumption. FRANCES A. HELLEBRANDT, RUBY H. TEPPER (by invitation) and ELIZABETH BROGDON (by invitation). Department of Physiology, University of Wisconsin, Madison. (Read by title.)

Within recent years attention has been drawn to the importance of further study of variability in metabolic rate so that single determinations might be reported accurately in terms of probability of normal. Using the method of closed circuit indirect calorimetry, we have made 227 five minute determinations of oxygen consumption on 3 normal adults in 48 experiments between April and July. These were conducted in the post-absorptive state, early in the morning, in a warm and quiet room after $\frac{1}{2}$ to 1 hour of bed rest. The subjects were trained to remain relaxed and motionless during 4 to 6 consecutive five minute runs following one another without interruption.

Of 81 determinations made on subject I, 56.78 per cent deviated from the mean oxygen consumption per minute by not more than ± 5 cc.; 80.23 per cent by not more than ± 10 cc.; 93.81 per cent by not more than ± 15 cc.; and 98.74 per cent by not more than ± 20 cc. The oxygen consumption per minute exceeded the mean value by more than 10 per cent in only 1 of 81 determinations.

The observations on the other two subjects were less strikingly uniform. For subjects II and III respectively they were 40.74 and 32.30 per cent of 81 and 65 observations deviating by not more than ± 5 cc.; 67.90 and 52.30 per cent by not more than ± 10 cc.; 86.42 and 73.84 per cent by not more than ± 15 cc.; and 95.06 and 93.84 per cent by not more than ± 20 cc. In every case the percentage deviation frequency histogram was skewed to the negative side but the range of positive variation was greater.

Metabolism of estrogenic hormones—in vitro studies. CARL G. HELLER, EMILY J. HELLER and ELMER L. SEVRINGHAUS (introduced by Walter J. Meek). Department of Medicine, University of Wisconsin Medical School, Madison.

Estradiol is inactivated when shaken with liver slices at 39 degrees for one hour. The optimum concentration of the estrogens is 0.3 microgram per cc. in 5 cc. of Ringer's phosphate buffer at pH 7.4 when 250 mgm. of tissue are added. The estrogens are assayed by the uterine weight method and compared with standard curves. Estradiol and estriol are *completely* inactivated by male and female rat liver, chick liver and rabbit liver. Rat lung, spleen and uterus caused no decrease in potency. Rat kidney decreased the activity slightly.

Inactivation by contact with liver slices was inhibited by first boiling the liver, by allowing the liver to autolyse or by adding NaCN to a concentration of 0.02 molar. Liver mice and liver juice, while causing some inactivation, were not nearly as effective in inactivating the estrogens as the tissue slice. Thus it may be inferred that the effective destroyer of estradiol is enzymatic in nature and that the process proceeds best when the intact liver cell is present. Adsorption was ruled out by injecting both the supernatant fluid and the whole tissue slices after maceration. The completeness of the inactivation rules out the possibility of conversion of estradiol to such estrogens of lower potency as estrone, estriol, estradiol benzoate or estradiol glucoside. Hydrolysis with both acid and alkali media has not caused an increase in potency of liver inactivated estradiol in experiments where destruction of free estrogen by the hydrolysis procedure was ruled out.

Liver tissue showed no increased oxygen uptake when estradiol was added as a substrate in a Warburg manometer. This does not necessarily rule out an oxidative mechanism being responsible for the destruction, since tissue respiration studies are less specific and less sensitive than the biological assay method used and since the known biological concentration threshold must be overstepped to reach the lowest theoretical point of sensitivity to increased oxygen uptake.

Contrary to our expectations liver tissue did not inactivate estrone. This might indicate a specificity of the responsible enzyme system. Spleen, kidney and uterine tissue caused an increase in potency when estrone was added. This suggests the possibility of the conversion of estrone to estradiol by these tissues.

The effect of nembital anesthesia on the response of dogs to cold. ALLAN HEMINGWAY. Department of Physiology, University of Minnesota, Minneapolis.

In practically all of the research which has been done to determine the parts played by central or peripheral (reflex) control of the temperature

regulating mechanism anesthesia has been used. The anesthesia has been necessary due to the acute nature of experiments required for these investigations. Since all anesthetics affect temperature regulation to varying degrees, some quantitative information concerning the depressive action on the various physiological components of the temperature regulating system is required. For this study the effect of application and removal of cold on the two systems, vasomotor and shivering, which protect against cold, have been investigated, first in the normal state and then under nembutal anesthesia.

Shivering was measured with a recording device which consisted of two platforms, one fixed beneath the trunk, forelegs, and head, and the other platform moveable and supported by piano wire. The moveable platform was located beneath the hind limbs. This device can record very small movements, such as respiration and occasionally the heart beat transmitted to the hind limbs. The hind limb flexion associated with shivering gives a well defined tracing which indicates slight and gross shivering movements. Incipient shivering consists of a few muscle twitches during inspiration. As the shivering becomes more intense the magnitude and number of muscle twitches increase but there is usually a pause at the end of expiration. Under normal resting conditions shivering occurs as a series of movements separated by pauses. These irregular intervals disappear during nembutal anesthesia.

Skin and rectal temperatures were measured during the onset of shivering and the disappearance of shivering when the cooled animal was heated by diathermy. A striking observation was the small amount of heat which inhibited shivering in both the normal and anesthetized animal.

Nembutal anesthesia depresses the rectal temperature at which shivering occurs but, due to vasomotor paralysis, the skin (particularly the ear) temperatures remain high. The order in which nembutal anesthesia depresses temperature regulation activities is in order of depression: 1, body temperature; 2, vasomotor tone; 3, shivering, i.e., shivering being least affected by the anesthetic.

A comparative study of blood flow in the splanchnic region with that in the femoral artery of the dog during exercise. J. F. HERRICK, J. H. GRINDLAY (by invitation), E. J. BALDES and F. C. MANN. Divisions of Experimental Medicine and Biophysics, The Mayo Clinic and Mayo Foundation, Rochester, Minn.

It is well-known that the blood flow to the muscle is increased markedly during exercise. The effect of exercise on the blood flow in the splanchnic region is not established so definitely. It is assumed by some investigators that the splanchnic vessels are constricted. Constriction of a blood vessel in situ does not necessarily imply a decreased blood flow. One might expect no change in flow or even an increase under certain dynamic conditions, i.e., a markedly increased cardiac output resulting in an increased blood pressure.

The stromuhr method of measuring blood flow permits observations in the dog during exercise on a treadmill. Dogs are trained for this study. Blood flow units are applied to the femoral and renal arteries, or to the femoral and superior mesenteric arteries under ether anesthesia employing the usual sterile technic. The effect of exercise on the blood flow in these vessels is recorded simultaneously after the dog has recovered from the

operation. In a separate study of the spleen the effect of exercise on its blood flow was likewise observed. As was expected, a marked increase of flow in the femoral artery was observed. The flow in this vessel may be increased as much as 300 per cent. No decrease of flow in the renal, superior mesenteric and splenic artery was observed. On the contrary, a definite increase occurred.

Afferent nerves excited by distention of the jejunum in dogs. R. C. HERRIN and W. J. MEEK. Physiology Department, University of Wisconsin Medical School, Madison.

In 1933, we reported that distention of jejunal fistulae in dogs produced a condition very closely resembling that of high intestinal obstruction (Arch. Int. Med. 51: 152). Section of all nerves in the mesenteric pedicle of the fistulae prevented the development of the experimental picture.

In order to identify the nerves responsible for the vomiting, anorexia and depression, jejunal fistulae were distended and various nerves were sectioned in 17 dogs. Bilateral vagotomy above the diaphragm did not prevent the three symptoms. Bilateral splanchnicotomy and lumbar sympathectomy prevented vomiting and to some extent the depression but anorexia appeared. Vagotomy, splanchnicotomy and section of the lumbar chain prevented the appearance of symptoms in 2 dogs but anorexia appeared in 3 dogs. Vagotomy, splanchnicotomy and lumbar sympathectomy permitted distention without any symptoms in 6 dogs. Unilateral denervation did not prevent symptoms.

An analysis of the total lipoids in hypothalamico-hypophyseal adiposity of white rats. ALBERT HETHERINGTON and ARTHUR WEIL (introduced by S. W. Ranson). Institute of Neurology, Northwestern University Medical School, Chicago, Ill.

During the course of experiments in our laboratory designed to repeat the work of Smith, who in 1930 reported adiposity in rats following the injection of chromic acid into the hypophysis, it was decided that detailed chemical analysis of the bodies of such obese rats would be helpful in understanding their metabolic disorder. Acetone-soluble fat, alcohol-soluble lipids, phosphorus, calcium, and iron determinations were accordingly carried out. The following table gives a comparison of operated rats and controls in percentages of controls. Litter A survived 195 days, litter B 277 days.

| LITTER | BODY WEIGHT | WATER | ACETONE EXTRACT | ALCOHOL EXTRACT | REST OF EXTRACT | P | Ca | Fe |
|--------|-------------|-------|-----------------|-----------------|-----------------|-----|-----|-----|
| A | +6 | -22 | +92 | +44 | -24 | -46 | -29 | -46 |
| B | +40 | -31 | +165 | +75 | -13 | -54 | -37 | -17 |

Quantitatively the total body fat, both acetone- and alcohol-soluble fractions, was found greatly increased in the fat animals. The proportion of cholesterol and its esters in the acetone-soluble fraction, and the saponification and iodine absorption numbers of this portion were practically the same in both operated and control rats, indicating no qualitative change had occurred in make-up of fat laid down in the bodies of the obese animals. Phospholipids were present in about equal amounts in all

animals, but the total alcohol-soluble fraction was increased in the fat rats, suggesting galactolipids might have been increased. Phosphorus, calcium, and iron were diminished in the body as a whole—phosphorus proportionately more than calcium, showing other body phosphorus depots besides the skeleton must have been partially depleted. It seems possible on the basis of the iron figures that the operated animals may have suffered from anemia as well.

Replacement of chlorides in tissues and body fluids of dogs by nitrates. EDWIN

P. HIATT (introduced by William R. Amberson). Department of Physiology, School of Medicine, University of Maryland, Baltimore.

Nitrates will substitute for chlorides in dogs much more readily than will sulfates. The administration of excess nitrates by mouth will cause chlorides to be eliminated in the urine, even when the serum chlorides are lower than the usual threshold value. If, at the same time, animals are fed a chloride-free diet, more than half of the total chloride of the animal can be removed in about a week. Such animals, with half of their chlorides replaced by nitrates, are apparently normal in their activities.

More chloride can be rapidly removed by plasmapheresis with an isotonic chloride-free solution made up with nitrate salts to which red blood cells and gum acacia are added. Another rapid method is to cause diuresis by slow intravenous injection of large quantities of nitrate solution.

The tissues lose chloride in direct proportion to the decrease in serum chloride. Unlike sulfates, nitrates will even drive chlorides out of central nervous tissue.

Circulatory changes in adults exposed to controlled environmental conditions.

FORD K. HICK (by invitation), ROBERT W. KEETON and NATHANIEL GLICKMAN (by invitation). Department of Medicine, University of Illinois College of Medicine, Chicago.

Normal subjects, two men and one woman, were observed in an air conditioned room in which the air currents were negligible and the temperature and humidity could be sharply controlled and regularly reproduced. Radiation effects were constant for each experimental condition. The subjects were nude, resting on a bed, and in the post-absorptive state.

Observations were conducted under four environments: cool (24.4°C. dry bulb; 10.6°C. wet), comfortable (28.9°C. dry bulb; 15.6°C. wet), hot dry (37.5°C. dry; 21.1°C. wet), hot moist (37.5°C. dry; 31.1°C. wet).

The normal subject showed no rise in basal cardiac output until his metabolism increased and his rectal temperature rose above 37.3°C. (99.2°F.).

Peripheral blood flow through the body as a whole was considered a function of conductance, i.e., the ratio of the total heat loss to the difference of rectal and mean surface temperatures. In the hot dry conditions this was about twice that in the comfortable zone, and was only slightly further raised in the hot wet condition. To study the blood flow through the arm, oxygen saturation of blood from a cubital vein was observed with figures of 48 to 64 per cent in cool zone; of 54 to 70 per cent in the comfortable zone; of 73 to 90 per cent in the hot dry zone, and of 81 to 93 per cent in the hot wet zone.

Blood volume studies showed a rise of 8 to 12 per cent in hot wet condition with an exposure of only 2 to 3 hours and no change in the comfortable and hot dry zones. Further physiological observations will be reported.

Contribution to local circuit theory. SAMUEL E. HILL. Rockefeller Institute for Medical Research, New York City.

Nitella cells were placed in a bath of NaCl solution of a concentration which would cause the appearance of spontaneous action potentials. The bath was so arranged that the action potentials could be recorded from three points. It is shown that the action potentials in three cells pull into phase or that the first cell to respond stimulates the others by its action current.

Observations on cats following pre- and postganglionic sympathectomies.

JOSEPH C. HINSEY, ROBERT A. PHILLIPS and KENDRICK HARE. Department of Physiology, Cornell Medical School, New York City.

Cats were observed over long intervals following pre- and postganglionic sympathectomies under controlled conditions (Am. J. Physiol. **123**: 101, 1938). Skin temperatures were recorded with Hardy radiometer. Incidental observations were made on skin galvanic reflexes and eye changes.

After preganglionic sympathectomy of left fore-limb (extradural section of thoracic ventral roots, 7 animals), preganglionic fibers regenerated in 36, 38 and 61 days as detected in skin temperatures (central paw-pads) and skin galvanic reflexes. After 229 days' regeneration, there was no deviation from unoperated limbs. Before regeneration, no sympathetic activity was seen in left pads. Skin resistance changes and galvanic reflexes were present 9 and 11 days before vasomotor activity was observed (2 animals).

Right abdominal trunk was removed from L1 through L7 and left trunk from L1 through L4 (3 animals). There was activity in left-hind-pads in 18, 45 and 68 days; in right pad in 40 days (1 animal). No return was observed on right side (2 animals).

Following removal of right thoracic chain (superior cervical ganglion through T8, stellate through T8, or stellate alone) in 11 animals, there was no evidence of regeneration to pad vessels after nearly a year. Pad temperatures remained about 30°C. or higher.

After left T2-T9 and T2-T10 ventral roots were cut, there was no Horner's syndrome and no sympathetic activity in left fore-pads. Before regeneration, pad temperatures were as high as in animals where T1 was included in operation.

After trunk was removed from stellate through T8, preganglionic fibers regenerated into remaining cervical trunks with return of sympathetic control of pupils, nictitating membranes and ear vessels. In 2 animals, 236 days after bilateral removal of thoracic trunks, right cervical trunks were removed from above superior cervical to below middle cervical ganglia. Right ear was then warmer than left, right Horner's syndrome returned but pad temperatures on right were unchanged. In these animals, this indicates middle cervical ganglion does not contribute significant numbers of postganglionic fibers to pad vessels.

With observations of other investigators, these findings emphasize importance of functional control of regeneration in work on sympathectomized animals.

*Nitrogen inhalation therapy for schizophrenia*¹. H. E. HIMWICH, F. A. D. ALEXANDER (by invitation), BASILE LIPETZ (by invitation) and J. F. FAZEKAS (by invitation). Departments of Physiology, Pharmacology, Anesthesia, Neurology and Psychiatry, Albany Medical College, Union University, Albany, N. Y.

Our studies of various therapies for schizophrenia have revealed that the underlying physiological change is a decreased cerebral metabolism. An analysis of blood drawn practically simultaneously from the internal jugular vein and femoral artery of patients receiving the insulin treatment disclose that hypoglycemia depresses cerebral oxygen uptake, so that the venous blood contains more oxygen though blood flow is not increased (Hall). In the absence of sugar, the chief foodstuff of the brain, cerebral oxidations can no longer be maintained. Loevenhart found that cyanide transitorily improved patients with schizophrenia. After the injection of cyanide in dogs we observed that the venous blood of the brain contains more oxygen, for cyanide inactivates cellular oxidase and thus prevents the combination of oxygen with substrate. Metrazol injections cause temporary arrest of respiratory movements. The blood, therefore, passes through the lungs without adequate oxygenation and the hemoglobin saturation of the arterial may fall to values as low as 40 per cent in patients treated for schizophrenia. In this manner the oxygen supply of the brain is rendered insufficient to satisfy its metabolic requirements.

Despite the beneficial effects of these treatments, objections have been made to their method of application. The danger of cyanide injection need not be emphasized. The insulin therapy is time consuming, requires a large staff, and thus limits the number of patients that can be so managed. Metrazol convulsions may cause fractures and dislocations but a more important impediment is the fear of the patients of this form of therapy. Because of these objections we are treating schizophrenia by subjecting patients to short periods of anoxia (about 5 min.) produced by the administration of nitrogen. Patients are so treated three times a week by one of us, F.A.D.A. for a period of about three months. The anoxia observed during nitrogen inhalation is more severe than that produced by metrazol and the hemoglobin saturation of the arterial blood may be reduced to 15 per cent. The results of this treatment in a small series of patients have been encouraging.

A comparison of the basal oxygen consumption of normal human beings as determined by standard commercial apparatus. FRED A. HITCHCOCK and FRANCES R. WARDWELL (by invitation). Department of Physiology, The Ohio State University, Columbus.

In an effort to estimate the degree of accuracy attainable with the common types of commercially manufactured closed circuit respiratory metabolism apparatus a comparative study has been made of the basal oxygen consumption of 25 normal human beings (11 males and 14 females) as determined by two or more types of such apparatus. The results obtained were then compared with the oxygen consumption of the same subjects as determined the same day by means of the Tissot-Haldane method. All tests were run under standard basal conditions.

Thirty comparisons were made between results obtained with the Bene-

¹ Aided by a grant from the Child Neurology Research (Friedsam Foundation).

dict-Roth apparatus and with the Tissot-Haldane technic. The average variation without respect to sign was 7.9 per cent, the range being from +34.4 to -15.5 per cent. In 63 per cent of the cases the variation was not more than 8 per cent. In 14 cases the Benedict-Roth gave higher figures, in 14 cases lower figures, and in the remaining two cases the results were identical. Twenty-seven comparisons were made with the Jones machine. Here the average difference between the two methods was 6.9 per cent, the range being from +40.5 to -12.4 per cent. In 63 per cent of the cases the variation was less than 6.9 per cent. In 7 cases the results were higher and in 20 cases lower than those obtained by the Tissot-Haldane method. There were 31 experiments run in which the results obtained with the McKesson apparatus were compared with figures obtained from Tissot-Haldane technic. In this case the average difference without respect to sign was 7.5 per cent. In 61 per cent of the cases the difference was no greater than the average and in 9 cases the results were higher, in 21 lower and in one identical with those obtained with the Tissot-Haldane method. A study of the internal resistance of all machines worked with and the effect of this factor on oxygen consumption has also been made.

We conclude that although the closed circuit apparatus tends to give results slightly lower than those obtained by the Tissot-Haldane technic this difference is so slight as to be unimportant. The more important sources of error are psychological factors in the subjects and errors of technic.

Effects of hypoglycemia and pentobarbital sodium on the electrical activity of the dog cerebral cortex and hypothalamus. HUDSON HOAGLAND, HAROLD E. HIMWICH, ELDRIDGE CAMPBELL (by invitation), JOSEPH E. FAZEKAS (by invitation) and ZAREH HADIDIAN (by invitation). The Biological Laboratories, Clark University, Worcester, Mass., and the Departments of Physiology, Pharmacology and Surgery, Albany Medical College, Albany, N. Y.

Electrograms have been recorded from the dog cortex (18 dogs) and from anterior and posterior hypothalamus (14 dogs) both with and without anesthetic under varying conditions of insulin hypoglycemia.

The effect of hypoglycemia on the cortical activity is similar to that in man, the alpha rhythms are slowed and the delta waves increase in prominence. In more prolonged hypoglycemia the cortical responses fail completely and are restored only an hour or so after injection of glucose. Both posterior and anterior hypothalamus show much stability compared to the cortex. No changes are noted until some time after failure of electrical activity of the cortex when a short period of hyperactivity of the hypothalamic centers may develop, followed later by failure just before death. Injected glucose restores hypothalamic activity some time before that of the cortex.

Nembutal, which reduces cortical respiration in vitro, produces cortical changes similar to those of hypoglycemia except that it considerably enhances the amplitude of alpha waves at the same time that it slows their rhythm. In contrast to this, no differences were seen in hypothalamic rhythms before and after nembutal anesthesia.

Our results show independence of responses from cortex, from a region near the supraoptic nucleus, and from the mammillary body region. The

latter two grid leads were approximately a centimeter apart. The anterior hypothalamic response, recorded directly by our method, gives records indistinguishable from those obtained by the Grinker technic in which a grid lead is embedded in the bone at the roof of the pharynx.

Changes (as much as 16-fold) in hydrostatic pressure of the cerebrospinal fluid are without effect on the cortical electrogram.

Correlation between the secretion of dyestuffs by the kidney and the molecular structure of these dyes. RUDOLF HÖBER and PRISCILLA M. BRISCOE (by invitation). Department of Physiology, University of Pennsylvania, Philadelphia.

The isolated Ringer perfused frog kidney, which is supplied from the renal portal vein with various well diffusible sulphonic acid dyestuffs, is able to select for secretory concentration only a certain number of those dyes. It has been found out that this selective ability is dependent upon the presence of a polar configuration of the dye molecules.

Cardiovascular and neuromuscular changes following intravenous injection of magnesium salts. H. E. HOFF, A. W. WINKLER (by invitation) and P. K. SMITH (by invitation). Laboratory of Physiology, Department of Internal Medicine and Laboratory of Toxicology and Pharmacology, Yale University School of Medicine, New Haven, Conn.

Magnesium sulfate in isotonic solution was injected intravenously into dogs using local anesthesia. Artificial respiration was employed when natural respiration failed. Blood pressure and electrocardiograms were compared with concentrations of magnesium in the serum. The first and most consistent change was a decline of blood pressure, noticeable before the concentration of magnesium had reached 5 m.-eq. per liter and continuing approximately along a straight line until death. The pulse rate rose early to reach a maximum at a concentration of 5 to 7 m.-eq. per liter, and thereafter gradually declined. Death from cardiac arrest occurred at concentrations usually ranging from 27 to 38 m.-eq. per liter, rarely higher. Occasionally cardiac arrest with simultaneous failure of natural respiration occurred at much lower levels, 15 to 17 m.-eq. per liter. One death only may have been due to ventricular fibrillation. The P-R interval increased progressively during injection, reaching as much as 0.30 sec. toward the end, when various types of heart block appeared. Calcium salts, simultaneously injected, had characteristic effects, but did not inhibit the action of magnesium. Respiratory arrest usually occurred at concentrations of magnesium ranging from 15 to 17 m.-eq. per liter. Certain preliminary experiments indicate that the velocity of conduction in peripheral nerves decreases and neuromuscular latency increases at still lower levels, 10 to 12 m.-eq. per liter.

The serious toxic effects of magnesium on the heart, since they appear at higher concentrations than do the changes in the nervous system, cannot be demonstrated unless the animal is kept alive by artificial respiration. The initial depression of blood pressure, however, precedes the effects on the nervous system, and may indeed be the only result of the injection of small quantities of magnesium.

A study of the character and the mechanism of the thirst induced by the intravenous injection of hypertonic salt solution. J. H. HOLMES (by invitation)

and M. I. GREGERSEN. Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

In approximately 200 experiments on 25 dogs, measurements were made of the amount of water drunk after the intravenous injection of 5, 10, 15 or 20 per cent sodium chloride (2.5 cc. per kgm.). Although the dogs showed considerable individual variations with respect to the amount and the rate of drinking produced by a given dose of salt, the results of repeated tests on the same animal were surprisingly constant. The time when water was given (up to 4 hours after the injection) had no effect on the total intake. Furthermore, in each animal the intake varied directly with the amount of salt injected.

The drinking response does not appear to be governed by the magnitude of the changes in the serum Na and Cl.

1. The increase in the serum concentration of Na or Cl may be identical in dogs in which the water intake differs widely.

2. When water is given, the serum electrolyte concentrations do not change for 15 minutes, yet drinking may be completed in 5 minutes.

3. Although the serum Na and Cl concentrations fall during the 4 hours following the injection, the drinking response remains unchanged during this period.

4. The subcutaneous administration of pitressin 20-30 minutes before the salt injection inhibits drinking for about 10 minutes in spite of the fact that in this instance the serum Na and Cl rise to much higher levels immediately following salt injection than when NaCl alone is given.

5. Water introduced through a gastric fistula 20 minutes before the salt injection inhibits drinking but does not prevent the rise in serum Na and Cl.

6. Under certain conditions dogs will drink large amounts of water although the concentration of serum Na or Cl may be greatly reduced (15-20 M.eq. below normal).

Changes in salivary flow may play an important part in the mechanism of salt thirst. In man, the intravenous injection of 300 cc. of 5 per cent NaCl causes an immediate fall in the salivary flow to one-tenth or less of its normal value. If, however, the subject drinks 500-800 cc. of water 20 minutes before the salt injection, thirst is slight or absent, and the salivary flow shows no significant variation.

Vasomotor responses in an organ with fixed volume. J. P. HOLT (by invitation) and HAMPDEN LAWSON. Department of Physiology and Pharmacology, University of Louisville School of Medicine, Louisville, Ky.

Changes in the peripheral resistance are usually the result of changes in the size of some portion of the peripheral vascular bed. Vasomotor phenomena are generally accompanied by changes in the total volume of an organ. To what extent these latter are an expression of the essential vasomotor change has not been thoroughly studied.

Blood flow changes in the hind limb of barbitalized or etherized dogs following section of the sciatic nerve or intra-arterial injection of nitroglycerine or epinephrine were measured by the direct venous outflow method and by a differential manometer method. Attempts were made to prevent the limb volume from changing by placing the limb in a tight plaster of Paris cast, or by wrapping the limb with porous bandage and placing it in a plethysmograph in which large negative pressures were

developed. In order to determine the effectiveness of these methods, the volume of the limb was measured by connecting the plethysmograph to a volume recorder through a mercury trap. In some experiments the plethysmograph was connected directly to the volume recorder which was placed in a chamber, in which pressure was lowered to that of the plethysmograph.

Sciatic nerve section and injection of epinephrine or nitroglycerine produced changes in blood flow through the limb ranging between 29 and 125 per cent. None of these procedures caused significant changes in the head of pressure (carotid pressure). They may, therefore, be regarded as effective through a change in the size of some portion of the vascular bed in the limb. Neither of our methods of keeping limb volume constant prevented these responses. In experiments in which fixation of limb volume did not materially affect the control flow, fixation of limb volume failed to modify the response to epinephrine or nitroglycerine.

These data show that significant flow changes through an organ, independent of the head of pressure, may occur without a change in the volume of the organ.

The effect of hemorrhage and other factors upon the blood flow. R. G. HORTON (introduced by A. G. Mulder). Department of Physiology, College of Medicine, University of Tennessee, Memphis.

Blood flow and blood pressure of dogs were studied. A Baldes-Herrick direct current thermostromuhr was placed on various arteries to register blood flow. There may be a temporary increased blood flow during hemorrhage followed by blood flow decreased below the pre-hemorrhagic level. There follows a slow increase toward normal during recovery. The proportionality of blood flow to blood pressure is changed under hemorrhagic conditions and reappears after the animal has recovered. Sodium hyposulfite can produce an enormously increased blood flow. Saline or Ringer-Locke intravenous injections following hemorrhage greatly increased blood flow and the greatest increase might occur in a period long after the injection and while blood pressure is decreasing.

Observations upon the effects of anoxia upon blood flow will be reported.

The effect of adrenalectomy on the seminal vesicles and prostates of castrated mice. EVELYN HOWARD. Johns Hopkins University School of Medicine, Baltimore, Md.

The maintenance of columnar epithelium in the seminal vesicles of castrated young mice has been shown to be associated in some degree with the presence of the adrenal X zone (Howard. *Am. J. Anat.* 1939, vol. 65). Such castrated mice were adrenalectomized, and maintained on a high NaCl intake. Of a group of forty mice, castrated at 21 days of age and adrenalectomized 2 to 4 days later, nine died within 5 days after adrenalectomy with loss of weight and were excluded because the interval was too short to expect sufficiently clear cut regressive changes in the vesicle. Twelve continued to grow at a continuous, approximately normal rate for 20 days, and were excluded as presumably having relatively large amounts of active cortical accessory tissue. Four showed large gains in weight but collapsed within 11 to 16 days and were found to have well maintained columnar epithelium in the vesicles. The remaining group of fifteen individuals showed small gain or actual loss of weight,

and died or were sacrificed, often in a state of collapse, 6 to 13 days after adrenalectomy. Of these, eight had columnar vesicle epithelium somewhat or little inferior to that of castrated mice with X zone bearing adrenals. In the remaining seven the vesicle epithelium had undergone clear cut regression: in contrast to that of castrates it was almost entirely reduced to the cuboidal state.

These findings suggest that the adrenal may be capable of secreting sufficient andromimetic hormone to be responsible for the anomalous maintenance of columnar epithelium in the vesicles of young castrated mice. However, to what extent and in what way this andromimetic action is related to the life maintaining action of the adrenal is not clear at present.

The medial cranial lobe of the prostate undergoes changes parallel to those in the vesicles. Hence it is indicated that the adrenal is a factor which may affect the development of the prostate.

Studies on the cardiac activity of human subjects during artificial hyperpyrexia. O. LEONARD HUDDLESTON, EDWARD J. BALDES and FRANK H. KRUSEN (by invitation). Department of Physiology and Pharmacology, University of Colorado School of Medicine, Denver, and Departments of Biophysics and Physical Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minn.

Electrocardiograms and optical polygrams were recorded simultaneously at various intervals during artificial hyperpyrexia in order to determine some of the changes in cardiac activity induced by the elevation of the body temperature. Central sphygmograms recorded from the subclavian or lower carotid arteries were employed to determine the duration of the components of the ejection phase. Jugular phlebograms supplied the time measurements for the various filling phases of the cardiac cycle. Observations were made on a series of 7 normal subjects and 4 patients who were receiving artificial fever therapy. Control polygrams and electrocardiograms were made before starting the fever treatment. Artificial hyperpyrexia was administered by means of an air conditioned fever cabinet. Pulse tracings combined with lead 2 electrocardiograms were recorded at approximately each degree elevation of body temperature up to and including 106° F. Analysis of the records obtained in our experiments permit the following general conclusions to be drawn:

1. The electrocardiograms showed a disappearance of sinus arrhythmia and a reduction of the conduction time (P-R interval).
2. The sphygmograms were modified in size, shape and composition. They became more triangular in shape and were often definitely 'spiked.' The dirotic notch became lowered and additional anacrotic, predierotic and postdirotic waves developed.
3. The phlebograms were altered in size, shape and contour. Atrial type phlebograms were immediately converted into an impact type as the body temperature began to rise.
4. The emptying time of the ventricles became progressively shortened from an average normal duration of 0.34 sec. to 0.18 sec. (53 per cent of the normal emptying time).
5. The total filling time of the ventricles became rapidly reduced and reached its lowest level (28 per cent of the total filling time) at 103.5° F.,

making the duration of the total filling time shorter than that of the total emptying time. This reduction of the filling time is believed to be contributory to the production of a temporary functional insufficiency during the induction stage of hyperpyrexia. Beyond 104.0° F. the duration of the filling phase again became longer than the emptying phase.

A study of pulse tracings and cardiac activity during artificial hyperpyrexia.

O. LEONARD HUDDLESTON, EDWARD J. BALDES AND FRANK H. KRUSEN (by invitation). Department of Physiology and Pharmacology, University of Colorado School of Medicine, Denver, and Departments of Biophysics and Physical Medicine, Mayo Clinic and Mayo Foundation, Rochester, Minn. (Demonstration.)

Polygrams consisting of pulse records of the jugular vein, subclavian artery or lower carotid artery were recorded optically by means of Frank segment capsules. These were superimposed upon lead II electrocardiograms which were recorded before and during the administration of artificial hyperpyrexia to human subjects. Hyperthermia was induced by means of an air conditioned fever cabinet. During an experiment a series of tracings were recorded at each degree of hyperthermia up to and including 106° or 107°F. (rectal temperatures). Photographs showing the apparatus, method of recording the pulse tracings, electrocardiograms, and artificial fever, samples of records obtained in the experiments, curves constructed from the polygram data and a table of results and conclusions constitute the demonstration.

Cord potentials in spinal shock: multiple volleys. JOSEPH HUGHES, WINIFRED B. STEWART (by invitation), and G. P. MCCOUCH. Pennsylvania Hospital for Nervous and Mental Diseases and Department of Physiology, University of Pennsylvania, Philadelphia.

As shown by Hughes and Gasser (Am. J. Physiol. **108**: 307, 1934) acute spinal cats showing no internuncial positivity are free from ipsilateral inhibition. Such preparations have been compared in cat, dog, and monkey in terms of the recovery of the internuncial potential after a preceding afferent volley, records being obtained from cord and from afferent nerve at each interval. Trains of five or six volleys have also been employed. In spite of minor differences in duration of the recovery curves (15 to 20 m.sec. in cat to 25 to 30 m.sec. in monkey) the general features have been similar in the three species. Where trains of several volleys were employed, the reduction was greatest in the second volley with recovery to an intermediate plateau level in the succeeding volleys. Such a picture accords with refractoriness rather than with inhibition or fatigue. In the monkey the internuncial recovery curve of the acute preparation is virtually identical with that twenty-five days after transection. Yet in the former, no reflex was obtained; in the latter, the reduction in the reflex component due to the second volley was proportional to the reduction of the second cord potential. In contrast to the reflex silence of the acute monkey stands the familiar reflex summation of 2 successive volleys in the acute cat and dog comparable to that of the monkey twenty-five days after transection. Where trains of five or six volleys are employed, even in the chronic monkey the reflex twitch tetanus ratio is far higher than in even the acute cat or dog. In such a monkey repetition of tetanus within a second develops a reflex tension of only about 20

per cent of that of the preceding response. We regard these results as evidence of the persistence of a considerable degree of shock to the motoneurons in even the chronic monkey.

Properties of growing nerve fibers. J. B. HURSH (introduced by H. S. Gasser). The Rockefeller Institute for Medical Research, New York City.

Saphenous and cervical sympathetic nerves of four-day old kittens were studied, and the same nerves of kittens at various ages up to three months, at which time the properties of the fibers become essentially those of the adult. The first elevation of the adult saphenous nerve is represented in the young nerve by two elevations, which later fuse. In the fastest fibers the velocity increases with the length of the leg and the diameter of the fibers. Thus increase in fiber length is accompanied by a proportionate increase in diameter and a constant conduction time.

Opportunity was afforded to examine the after-potentials of immature A fibers conducting at B velocity (12-4 m.p.s.). The positive after-potential had an A duration and a magnitude which, although slightly larger than that of A, was much smaller than that of B. Clearly the after-potential retained its A identity and was not determined by fiber size or other features characteristic of B fibers.

Action potentials of young cervical sympathetic nerves (unless depressor fibers are present) contain a single elevation at C velocity. This condition obtains until 15-20 days after birth, when a portion of the fibers undergo a three- to fourfold increase in velocity and the B elevation appears. As at 20 days many nerve fibers are myelinated, the increase may be related to the completing of the myelination process. The B fibers function before they are myelinated.

The spike durations in the fibers that will have the fastest velocities in adult nerves have a value falling in the adult range (0.4-0.5 msec.) as soon as the velocities in the immature fibers reach 20 m.p.s. Below this velocity the durations appear slightly longer, but the measurements are more uncertain.

The refractory period of young fibers varies as the velocities change. A fibers conducting at velocities from 5-20 m.p.s. have a refractory period which progressively decreases from about 1.4-0.5 msec. At velocities greater than 20 m.p.s., the refractory period remains constant between 0.5-0.4 msec.

The heart configuration of young athletes. K. HYNEK (by invitation), JIŘÍ KRÁL (by invitation), J. BAŠTECKÝ (by invitation) and FRANCES A. HELLEBRANDT. Charles University, Prague, and Department of Physiology, University of Wisconsin, Madison. (Read by title.)

Teleroentgenograms were made on a random sample of 675 adolescent boys competing in the Xth Pan-Sokol Festival in Prague. The subjects, numbered 167, had been participating in Sokol activities for an average of 3 years and took part in a four activity event for which they had been training for approximately six months.

Inspection of the frontal view cardiac silhouettes showed a normal configuration in 56 per cent. In 5 per cent the contour was mitral as diagnosed by the disappearance of the concavity at the junction of the left cardiac border with the great vessels, dilatation of the pulmonary artery,

prominence of the left auricular salient and bulging of the right cardiac border. In 38 per cent the type was transitory. In no subject did medical examination reveal organic disease.

A mitral configuration had previously been found (K. Hynek, J. Král and J. Baštecký. *Časopis Lék. Českyh.* **72**: 1113, 1933) in 14 per cent of 176 boys between the ages 17 and 18 competing in the IXth Sokol Festival, and a transitory type in 54 per cent. The question which arises is whether these configurations are physiological or related to the participation in exercise. The lower incidence of "abnormal" contours in the 15 and 16 year old boys suggests years of exercise as a dominant factor, but there was no significant relation between configuration, athletic history or excellence of performance.

The work-performance of adrenalectomized rats treated with corticosterone and chemically related components. DWIGHT J. INGLE (introduced by F. D. W. Lukens). George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

When the normal rat is anesthetized with phenobarbital-sodium and the gastrocnemius muscle stimulated to lift 100 grams three times per second, it is able to work continuously for several days. Rats which are made to work under identical conditions immediately following adrenalectomy are able to work in a normal manner for a few hours, but the capacity to work is lost within the first 24 hour period. When adrenalectomized rats are treated with cortical adrenal extracts or with corticosterone, the performance of work is sustained at a normal level.

Using the first 24 hours following adrenalectomy as the test period, we have compared the work performance of untreated adrenalectomized animals and normal animals with that of adrenalectomized animals treated with 1, compounds isolated from the adrenal cortex; 2, androgenic substances 3, oestrogenic substances, and 4, progesterone.

Compounds which are closely related to corticosterone in structure, such as 11-dehydro-corticosterone, 17-oxy-corticosterone, 17-oxy-11-dehydro-corticosterone, and desoxy-corticosterone, each possess the property of increasing the capacity of adrenalectomized rats to work. Oestrogenic and androgenic substances and progesterone were administered to adrenalectomized rats in large amounts, but the work performance of these animals did not differ from that of untreated animals.

*The influence of anoxia on the pH in the brain.*¹ R. C. INGRAHAM and E. GELLHORN. Department of Physiology, College of Medicine, University of Illinois, Chicago.

Experiments were carried out on amyralized dogs which inhaled for various periods of time oxygen-nitrogen mixtures from Douglas bags while the pH of the cortex of the brain was determined by the glass electrode according to Dusser de Barenne, McCulloch, and Nims (1937). It was found that oxygen deficiency is accompanied by a reversible increase in pH. The effect is observed with as high an oxygen concentration as 13 per cent and increases with falling oxygen concentration. Similar effects were observed in muscle. Even with inhalation of commercial nitrogen no increase in acidity in the brain is observed. Since all experiments were carried out with artificial respiration the increased alkalinity of the tissue during anoxia is not the result of a respiratory

¹ Aided by a grant from The John and Mary R. Markle Foundation.

adjustment but the direct effect of a decrease of the rate of oxidation in the tissues.

Observations upon the blood sugar and upon the maintenance of life with persisting polyuria, without cortin, in adrenalectomized cats with diabetes insipidus. W. R. INGRAM, C. A. WINTER (by invitation) and E. G. GROSS. Departments of Anatomy, Physiology and Pharmacology, State University of Iowa, Iowa City.

Because cats with hypothalamic lesions of the type used in producing polyuria sometimes show signs of anterior lobe suppression as manifest by increased sensitivity to insulin, and since hypophysectomized adrenalectomized animals are said to have very short survival periods, it was necessary to determine if the comparatively brief survivals of adrenalectomized polyuric cats are due to anterior lobe involvement. Insulin sensitivity tests on 10 d.i. cats before adrenalectomy showed 5 to be hypersensitive. D.i. adrenalectomized cats of previously normal sensitivity to insulin may have survival times as short as the hypersensitive cats. Pitressin administration prolonged the survival time of both types, on the average, but under such treatment the hypersensitive animals succumbed about 2 days before the others. Two polyuria cats of normal pre-adrenalectomy insulin sensitivity showed terminal blood sugars of 70 and 83 mgm. per cent. Similar animals on pitressin treatment had terminal blood sugars of 67 to 96 mgm. per cent. Two animals hypersensitive to insulin before adrenalectomy showed terminal blood sugars of 46 and 88 mgm. per cent. Similar cats on pitressin had terminal blood sugars of 55 to 86 mgm. per cent.

Prolongation of the survival period of d.i. adrenalectomized cats without the use of cortin has been attempted by administration of additional salt in the food and drinking water. Addition of one grain extra NaCl failed in several instances to increase the survival time, but one cat on such treatment lived 13 days after adrenalectomy. D.i. cats given 0.5 per cent NaCl solution to drink plus 1.5 grams NaCl and 1.25 grams Na citrate in the food develop enormous polyurias. After bilateral adrenalectomy such cats survive in good health for a considerable time—the limits of which are unknown. After discontinuing the added salts, the water exchange falls rapidly, the cats decline quickly and die in one or two days, the blood chlorides just before death being of normal, or above normal, concentration. The maintenance of marked polyurias in these animals offers evidence that cortin is not essential for the occurrence of the polyuria of diabetes insipidus in the cat.

Effect of oral administration of desiccated hog bile on the bile acid composition of human and canine fistula bile. J. LOGAN IRVIN (by invitation), CHARLES G. JOHNSTON and CARL E. ANDERSON (by invitation). Department of Surgical Research, School of Medicine, Wayne University, Detroit, Mich.

Differential bile acid analyses were carried out for total bile acids, cholic acid, the "desoxycholic acid group," total conjugated bile acids, and bile acids conjugated with taurine on desiccated hog bile and on human and canine fistula bile by methods involving several modifications of the usual procedures.

Drainage bile was collected postoperatively from several human pa-

tients following removal of common duct obstructions. The concentrations of all the bile acid fractions were very low immediately following operation but increased throughout the recovery period. During the entire collection period, the total bile acid concentration was greater than the concentration of cholic acid, and the increase in the "desoxycholic acid group" of bile acids was more rapid than the rise in cholic acid concentration. Administration of desiccated hog bile by mouth produced a marked increase in concentration of all the bile acid fractions, but the increase in the "desoxycholic acid group" was greater than that of cholic acid. Unconjugated bile acids were present at all times during the period of drainage, but there was a decided increase in the ratio of conjugated bile acids to total bile acids following administration of hog bile. This increase was largely in the bile acids conjugated with glycine.

Individual variations were noted in the total bile acid secretion and in the proportions of the various bile acids secreted by the dogs studied. The total conjugated bile acids were accounted for principally as tauroconjugated acids. Administration of desiccated hog bile by mouth produced marked increases in the volume of bile flow and in the total bile acid secretion, but the concentration of bile acids was not changed appreciably. The principal increase in the bile acid secretion was in the "desoxycholic acid group." Isolations and analyses indicated the presence of hyodesoxycholic acid in the dog bile following hog bile ingestion. This bile acid was included in the "desoxycholic acid group" by our analytical procedure. Following the feeding of hog bile, the tauroconjugated bile acids no longer accounted for all the total conjugated acids indicating the secretion of some bile acids conjugated with glycine.

The respiratory tolerance of some Pennsylvania fish. LAURENCE IRVING, EDGAR C. BLACK (by invitation) and V. SAFFORD (by invitation). Edward Martin Biological Laboratory, Swarthmore, Pa.

The work of Fry and Black (*Anat. Rec.* **72**: Supplement p. 47, 1938) on Canadian fish has been extended to the fresh-water fish of Pennsylvania. CO_2 reduces the oxygen combining power of blood of some fresh-water fish much more than it does in mammalian blood. In consequence it would be expected that small pressures of CO_2 in the water would make it difficult for adequate oxygenation of blood to occur in the gills. Trial of 12 species showed that for each species a characteristic pressure of CO_2 was fatal. The lethal pressure of CO_2 diminished with decreasing pressure of O_2 . At the time of death, the pressure of O_2 plotted against pressure of CO_2 gave a curve characteristic of each species of fish. Curves for the several species formed a series. Species from the same habitat gave curves adjacent to each other.

In this manner the tolerance of various fish to external respiratory conditions may be compared on a quantitative basis. The tolerance curves for various species show a relative sensitivity to CO_2 in accordance with the expectation from the characteristics of the blood, so far as the latter are known. The properties of blood represent the capacity for respiratory transport of the first stage of internal respiration. The P_{O_2} and P_{CO_2} relations of the external respiratory medium show the same factors in the external environment. Other factors than P_{O_2} and P_{CO_2} , such as temperature influence respiratory tolerance, and their influence may be examined by reference to their effect upon blood.

The comparison of respiratory tolerance with properties of blood in fish is significant in regard to distribution. Inasmuch as fresh-water fish are subject to far greater variations in respiratory conditions than are air breathing animals or marine fish, the comparison is also interesting physiologically. Other factors than P_{CO_2} and P_{O_2} undoubtedly affect respiratory tolerance, and at least one species in Ontario is distinctly more sensitive than the same species in Pennsylvania.

Certain features of the monkey's uterus during labor. A. C. IVY, CARL G. HARTMAN and A. KOFF (by invitation). Carnegie Laboratory of Embryology, Baltimore, Md., and Department of Physiology, Northwestern University, Chicago, Ill. (Motion picture demonstration.)

The film shows active spontaneous contractions of the monkey's uterus exposed under anesthesia during labor. The cervical sphincter, the relative inertia of the placental sites, the contraction of the upper uterine segment after birth of the fetus, the evacuation of the placenta by the Credé method, and the contraction (brachystasis) of both upper and lower uterine segments after the completion of labor are to be observed.

Variations in blood pressure with skeletal muscle tension (action-potentials) in man. III. *The influence of brief voluntary contractions.* EDMUND JACOBSON. Laboratory for Clinical Physiology, Chicago, Ill.

Evidence that a relationship exists between the general state of skeletal muscle tension and the blood pressure in man has been reported previously (Proceedings, Am. J. Physiol. **115**: 123: 1936, 1938). Whether pressure tends to increase during brief voluntary contractions has not been determined clearly. Although vigorous exercise is known to increase the pressure, it has been stated that mild exercise does not.

For quantitative recording of action-potentials in electrodes in skeletal muscles, two amplifier-string-galvanometer assemblies were used as previously, capable of measurements to a fraction of a microvolt within the frequency range 30-4000. Of 15 subjects employed, blood pressure was normal in 11, six of whom had received training to relax, while the five others were athletes. The four remaining subjects showed chronic high blood pressure, but were being trained to relax.

The subject as a rule has been lying quietly for fifteen minutes or more on a couch, carefully shielded. Upon signal, he clenches the right fist (or engages in other contraction elsewhere) for several minutes or more while instructed to keep relaxed so far as possible elsewhere. Upon a second signal he relaxes promptly. Blood pressure is recorded graphically. Under the conditions of the experiment, it is evident that emotional factors are reduced to a minimum or absent altogether.

While the results secured are not positive in all instances, they seem to reveal a trend distinctly. *The trend is obscured if the subject fails to be well relaxed preceding the contraction.* During clenching of the right fist, in the eleven subjects with normal pressure, systolic pressure was moderately but distinctly increased in 64 and diastolic pressure in 63 out of 70 determinations. Increases occur also in the vascular hypertensive subjects. In all subjects more strongly positive results are often secured if and when additional muscle groups participate in the contraction—showing a sort of "cumulative mass action."

Accordingly the present findings lend further support to the view that

the blood pressure levels vary to a determinable extent with the magnitudes of contraction in the various skeletal muscles.

The effect of changing peripheral resistance on pulse volume and pulse pressure. K. JOCHIM, R. BALLIN and J. RANSOHOFF, JR. (introduced by L. N. Katz). Cardiovascular Department, Michael Reese Hospital, and the Department of Physiology, University of Chicago, Ill.

Pulse volume and pulse pressure changes in a segment of aorta were studied in both the artificial circulation machine and the living dog under conditions of varying peripheral resistance. In the circulation machine, both rubber "aortas" and freshly excised dog's aortas were used. The pulse pressures were recorded with Hamilton manometers, and a specially constructed oncometer was employed to record optically the simultaneous pulse volume changes.

Keeping other factors constant, an increase in peripheral resistance caused an increased pulse volume in all three types of experiments throughout the physiological range of pressures studied. This indicates a redistribution of peripheral flow, a greater proportion of the total now taking place in diastole. It is to be noted that peripheral resistance is not a static attribute of the physical system used, but is a dynamically varying factor, depending for its effective value on a power function of the velocity of flow. Since a mechanically increased peripheral resistance, with minute volume flow constant, causes an increased linear velocity of flow in the periphery, the dynamic changes in effective resistance are definitely altered. This factor must be taken into consideration in explaining our results. A complete analysis of the dynamics of an elastic system requires a correlation of five important factors, viz.: 1, volume pulse; 2, pressure pulse; 3, velocity pulse in the periphery; 4, the distensibility curve of the elastic system, and 5, cardiac stroke volume used. In this study we have correlated the volume pulse with the pressure pulse and the elastic distensibility curves. Although the pulse volume always increased when peripheral resistance was raised, the pulse pressure either increased or decreased, depending on the elastic properties of the system used. Correlations with velocity pulse are now being studied.

*Some improvements on the electromagnetic flowmeter.*¹ K. JOCHIM (introduced by L. N. Katz). Cardiovascular Department, Michael Reese Hospital, Chicago, Ill. (Demonstration.)

The electromagnetic flowmeter developed in this laboratory (Kolin. This Journal **122**: 788, 1938) provides a method for accurately recording the velocity pulse in an unopened blood vessel. The advantages of the instrument are: 1, the ability to record rapid cyclic variations in blood velocity in an unopened vessel; 2, the ability to record the direction of blood flow; 3, the determination of mean blood flow by integration of the velocity curve, and 4, a linear calibration curve. The improvements in the method to be demonstrated are: 1, a much smaller and more convenient permanent magnet to supply the magnetic field; 2, a special amplifier with a much higher gain, and 3, much smaller and more convenient

¹ Aided by a special grant to L. N. K. from the American Association for the Advancement of Science.

non-polarizable electrodes incorporated with the sleeve used to keep constant the diameter of the blood vessel at the point of flow measurement.

Free choline and phospholipid of bile. CHARLES G. JOHNSTON, J. LOGAN IRVIN (by invitation) and CLARENCE WALTON (by invitation). Department of Surgical Research, School of Medicine, Wayne University, Detroit, Mich. (Read by title.)

The literature does not contain satisfactory evidence for the presence of lecithin in bile. Jones and Sherberg in 1937 reported that neutral fat and lecithin are either absent from gall-bladder bile of the ox, the hog and the dog or are present in very minute quantities.

In the present investigation, human and canine hepatic and gall-bladder bile and gall-bladder bile of the hog were extracted by an ether-alcohol procedure. Phosphate analyses and choline determinations by the Reinecke salt method after hydrolysis were carried out on the extract. Analyses for phosphate and choline were made in the same manner on the watery bile fraction from the extraction. Difficulties in the complete extraction of the lipoids of bile were encountered. Such difficulties have been reported by others and possibly are due to the formation of choleates with the bile salts. Fatty acids, choline, and phosphate were found in the ether-alcohol extracts of all the biles studied. Choline and phosphate were present in approximately equimolar quantities indicating that the phospholipid may be lecithin, sphingomyelin, a mixture of the two, or some previously undescribed compound containing choline. By extraction, the phospholipid contents of the various biles, calculated as milligrams of lecithin per 100 cc. of bile on the basis of the phosphate and choline content of the extracts, were for human gall-bladder bile (190), human fistula bile (60), dog gall-bladder bile (250), dog fistula bile (30), hog gall-bladder bile (290). These figures are the averages of a number of determinations. The combined-choline and combined-phosphate in the ether-alcohol extracts represent only a small part of the total quantities of these materials in the biles studied. A small amount of uncombined choline was present in all the fresh bile samples analyzed by our procedure. The amount of uncombined choline increased when the bile samples were stored in an ice box for several days before analysis. Desiccation of bile by the "lyophile" process produced an increase in the quantity of free choline at the expense of the combined-choline.

Solubility of cholesterol in gall-stone solvents. K. K. JONES and MARIE LORENZ (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

It is the common hypothesis that cholesterol is the ingredient of gall stones that binds the various constituents into a solid concretion. Human stones vary widely in quality even when taken from the same gall-bladder. Dolkart, Jones, and Brown (Arch. Int. Med. 62: 618, 1938) have shown that this variability can be controlled by reducing a large number of gall stones to a fine powder and pressing this into pellets of uniform size and consistency.

The results obtained by using pellets of cholesterol and pellets of gall stone powder confirmed the hypothesis that the disintegration of gall stones by various solutions is due to the solution of cholesterol. The

results show very definitely that lauric and myristic acid are 2 to 3 times better solvents than other fatty acids and in 1 per cent concentration are roughly 40 to 50 times better solvents than the bile salts alone. Bile salts added to fatty acid solutions do not improve the solvent action of the latter in vitro at an alkaline reaction.

Habituation to the use of a laxative in man. FREDERIC T. JUNG and BERTHA L. ISAACS (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

In the course of a study concerned primarily with the utilization of vitamin A in man, a group of volunteers were persuaded to take an oily laxative daily for several months. As an incident to this study it was noted that the subjects acquired some tolerance to the laxative. Preparations were therefore made for a study of anticipated withdrawal symptoms. The purpose of this paper is to report the data obtained.

Aside from a large number of controls, there were 24 students who took the laxative daily for periods varying from 91 to 131 days. Their ages ranged from 21 to 34. The medication consisted of two tablespoonfuls daily of an emulsion containing 65 per cent of liquid petrolatum in agar. Signs of habituation were seen when the symptoms of the first week were compared with those of the second. The most striking change involved the colicky pains, flatulence, and leakage of oil which were troublesome during the first week and markedly subsided during the second. An extreme diarrhea in one subject subsided without his discontinuing the laxative. Before the medication was begun, periodic tests of dark adaptation were started on the subjects and on a control series. It was hoped that this procedure would detect subclinical evidence of hypovitaminosis A, but no statistically convincing difference was obtained.

Contrary to expectations, no striking withdrawal symptoms followed the discontinuation of the laxative. Thirteen subjects submitted complete and comparable series of daily weighings for a period of 5 days before and 7 days after discontinuation. The averages were 154.8, 154.6, 155.0, 155.0, and 154.6 pounds respectively for the 5 days before, and 154.6, 154.9, 154.7, 154.6, 154.8, and 154.6 pounds for the 7 days after discontinuation. The records show no marked change in defecation habits during the readjustment period.

Behavior of dogs after complete temporary anemia of the brain. HERMAN KABAT and CLARENCE DENNIS (introduced by M. B. Visscher). Department of Physiology, University of Minnesota, Minneapolis.

Sudden complete anemia of the head has been produced in unanaesthetized dogs (Proc. Soc. Exper. Biol. and Med. **38**: 864, 1938). Brain anemia was maintained for periods of two to nineteen minutes in different animals, after which the animals were resuscitated and kept alive for one to several weeks.

A dog which survived for nine days after nineteen minutes of anemia exemplifies the effects of severe anemia of the brain. It appeared comatose throughout the period of survival and had no apparent sensations of vision, hearing, smell and taste. It could not stand but lay on its side, with extensor tonus only moderately increased. It showed frequent periods of high-pitched vocalization and rhythmic running movements involving all four limbs. Temperature regulation was markedly impaired

and no fever was produced by injection of typhoid vaccine. Brain stem reflexes were normal, including the response of the respiratory mechanism to CO_2 . The carotid sinus and aortic arch reflexes were hyperactive. No improvement was noted during the period of survival. Examination of the brain revealed: No nerve cells in neo-cortex, corpus striatum and parts of thalamus; no Purkinje cells in cerebellum; other parts histologically normal.

Similar behavior was exhibited by dogs surviving ten minutes of anemia. Periods of anemia of eight minutes or less resulted in coma for eighteen to thirty-six hours, followed by gradual recovery of sensation and intelligent behavior. The outstanding symptom in these animals after the first week was an ataxia of clinically cerebellar type. Puppies appear to be less severely affected by brain anemia than adult dogs.

The influence of the adaptation syndrome on blood volume and on the chloride distribution between erythrocytes and plasma. S. KARADY (by invitation), H. SELYE and J. S. L. BROWNE. McGill University Clinic, Royal Victoria Hospital, and the Department of Anatomy, Histology and Embryology, McGill University, Montreal, Canada.

It has been found that during adaptation to various damaging stimuli the organism responds with certain characteristic symptoms which have been described under the name of the "general adaptation syndrome." Among these a decrease in the total blood chlorides and blood volume was found to accompany the first acute stage of the syndrome while later when adaptation had occurred an increase in blood volume and total chlorides took place under the influence of the same stimulus. In the present investigation the distribution of chlorides between plasma and erythrocytes in adult "hooded" rats during adaptation to muscular exercise, cold and subcutaneous injections of formaldehyde was investigated. The blood volume, haematocrit value and haemoglobin concentration were also determined. In most cases red blood cell chlorides were determined directly as well as being calculated from the chloride concentration in plasma and whole blood and the haematocrit reading.

After 24 hours' treatment when the total blood chlorides and blood volume were low the chloride concentration in the red cells had decreased markedly (from 170 to 95 mgm. per cent) after formaldehyde, less markedly after cold, while after exercise, a stimulus which does not decrease the total blood chlorides during the acute stage, the chloride concentration of the red cells increased. The plasma chloride increased but slightly after formaldehyde, as well as after cold and remained unchanged after exercise. Blood volume decreased while the haematocrit and haemoglobin increased in all cases. All these changes were reversed within 2 to 24 hours after the stimulus had been discontinued in the case of cold and formaldehyde, while in the case of muscular exercise which leads to an increase in red blood cell chlorides from the beginning this change was simply intensified so that at this time there was an increase in red cell and whole blood chlorides, a decrease in haematocrit and haemoglobin values while the blood volume was at or above normal after all three stimuli. During the resistant stage after twelve days of treatment such an inverse response was seen even if the determinations were made immediately after discontinuing the stimulus. If at this stage the animals were treated with sub-lethal doses of the stimulus to which they had been

adapted they responded with no change or an increase in total blood and erythrocyte chlorides even though their blood volume might decrease considerably and judged by their appearance they were in severe shock.

*The effect of adrenal cortical extract on the carbohydrate and protein metabolism of the rat.*¹ B. KATZIN (by invitation) and C. N. H. LONG. Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven, Conn.

When fasted normal, adrenalectomised, or hypophysectomised rats are injected with adrenal cortical extract in doses of 1 cc. for 12 hours there occurs a marked increase in the liver glycogen and the blood glucose; the muscle glycogen is, however, not affected. At the same time there is found an increased excretion of urinary nitrogen sufficient to account for this newly formed carbohydrate (table 1).

TABLE 1
All values—milligrams per 100 grams rat

| | LIVER GLYCOGEN | MUSCLE GLYCOGEN | GLUCOSE IN BODY FLUIDS | EXTRA URINE NITROGEN |
|--------------------------------------|-------------------|--------------------|------------------------------|----------------------------|
| | | | | mgm. |
| Normal controls (12)..... | 7 ± 1 | 253 ± 15 | 37 ± 2 | |
| Normal injected (7)..... | 77 ± 9 | 269 ± 15 | 53 ± 2 | 46.6 |
| Hypophysectomised controls (8)..... | 3 ± 1 | 155 ± 12 | 25 ± 3 | |
| Hypophysectomised injected (11)..... | 26 ± 5 | 162 ± 12 | 51 ± 3 | 43.2 |
| Adrenalectomised controls (4)..... | 2 ± 0.4 | 179 ± 11 | 15 ± 1 | |
| Adrenalectomised injected (4)..... | 80 ± 8 | 198 ± 6 | 47 ± 1 | 38.5 |

When normal rats fasted 24 hours are fed glucose and killed four hours later, those injected with cortical extract (total dose 5 cc.) exhibit a depression of the R. Q. (six controls 0.86 ± 0.005 ; six injected 0.78 ± 0.008). This decreased proportion of carbohydrate oxidation is accompanied by an increased deposition of the absorbed glucose as liver glycogen and by an increased glucose level in the blood and tissues while the effect on the muscle glycogen is less marked (table 2).

TABLE 2
Percentage distribution of glucose absorbed—4 hours after feeding

| | GLUCOSE ABSORBED | MUSCLE GLYCOGEN | LIVER GLYCOGEN | BLOOD AND TISSUE GLUCOSE | OXIDIZED | RECOV- ERY |
|---|---------------------|--------------------|-------------------|--------------------------------|------------|---------------|
| | mgm/100 g. | per cent | per cent | per cent | per cent | |
| Controls (6)..... | 766 ± 16 | 17.5 ± 1.5 | 16.4 ± 1.2 | 2.6 ± 0.4 | 48.3 ± 2.0 | 84.8 |
| Injected with corti- cal extract (6).... | 683 ± 29 | 24.0 ± 2.7 | 24.0 ± 0.9 | 5.5 ± 0.8 | 25.7 ± 2.2 | 79.2 |

These results indicate that the adrenal cortical hormone has a direct effect on carbohydrate metabolism, stimulating in the fasted rat an increased formation of glucose from protein and in the fed animal decreasing the utilization of glucose.

¹ This work was assisted by grants from the Committee on Research in Endocrinology, National Research Council and the Fluid Research Fund, Yale University School of Medicine.

A further observation on atonia following transection of the brain stem through the pons. ALLEN D. KELLER. Department of Physiology and Pharmacology, University of Alabama School of Medicine, University.

I reported last year reasons for believing that the atonia and hyporeflexia encountered following transection of the brain stem through the middle of the pons is not due to functional infringement upon the vestibular nuclei. The crucial test in this regard would be long chronic preparations. The longest period that animals with complete transections at this level have been maintained is from 10 to 18 days; the experiments always being terminated by pneumonia. The following experiment, however, serves the criteria of chronicity at least in part. Hemisection of the brain stem through the pons on one side preceded that on the other side from 1 to 6 months. Whereas the first hemisection did not produce atonia, the second hemisection precipitated bilateral atonia exactly as though both hemisections were made simultaneously.

The hyporeflexia in these preparations is characterized by the absence of righting, standing, cross extension, and scratch reflexes. The withdrawal reflex, when present, is weak. The knee jerks are present immediately after operation, and frequently *the response is bilateral*, i.e. tapping the tendon on one side elicits jerks on both sides.

The level of transection requisite for precipitating atonia corresponds with the level at which a hemisection precipitates contralateral unilateral overflexion. Therefore, the question is raised as to whether overflexion is due to the unilateral release of the same mechanism which, when released bilaterally, produces complete atonia, or whether the atonia simply masks bilateral overflexion. It likewise seems just possible that both overflexion and atonia are due to the release of the mechanism, or mechanisms, which respond similarly when activated by intracerebellar stimulation. It is to be borne in mind that the immediate foregoing statements are purely problematic. (This paper was illustrated by a moving picture demonstration.)

Apparent motor apraxia in the monkey. ALLEN D. KELLER. Department of Physiology and Pharmacology, University of Alabama School of Medicine, University. (Read by title.)

A characteristic unilateral contralateral deficit which is similar, but may or may not be identical, to clinical motor apraxia is present in the monkey following radical removal of the cerebral cortex lying posterior to the anterior extent of the post central gyrus. The animals fail to execute *purely voluntary* movements, such as picking food off the floor or passing the hand through a hole in a screen, in order to obtain food (with or without the uninvolved hand immobilized); the same procedures being executed freely by the uninvolved hand. Ordinary paralysis is not present, as evidenced by the lack of asymmetry in the facial muscles and the free use of the limbs in more or less involuntary associate movements, such as walking, climbing, scratching, certain grooming movements, swatting flies, and manipulation of the food at the mouth after it has first been picked up by the other hand, or if this hand has been immobilized, by the mouth. The syndrome is precipitated by either right or left ablations. Removal of the cortex lying posterior to the anterior third of the parietal lobe renders the animal definitely right- or left-handed, but a full

blown deficit does not persist until the post central gyrus is encroached upon (particularly dorso-medially).

Although this syndrome may be due to a purely sensory or motor deficit, or a combination of both, I wish to raise the question as to whether we are not dealing here primarily with a true *unilateral* "intellectual deficit" entirely equivalent to motor apraxia as it is encountered in man. Necropsy material is at hand on two specimens only, the most conspicuous finding being the degeneration of the lateral cerebro-pontine fibers.

The effect of variations of atmospheric oxygen concentration upon the metabolism of tubercle bacteria. WALTER KEMPNER (introduced by G. S. Eadie). Department of Medicine, Duke University, Durham, N. C.

The respiratory metabolism of 15-20 days old cultures of tubercle bacteria H 37 grown on Steenken-Smith medium was measured at 37.5°C. manometrically by the Warburg method in M/80 glycerol-M/50 phosphate (pH 7.4) solution at 14 different oxygen concentrations between 0.1 and 100 per cent of one atmosphere (oxygen partial pressures of 0.76 to 760 mm.Hg). Two and one-half liters of N₂/O₂ mixtures of different concentrations, prepared over mercury, were used for saturation. Insufficient oxygen diffusion from gas space to cell surface was ruled out as a limiting factor by determinations at various shaking speeds (90-220 oscillations per minute). The experimental periods were of 2 to 24 hours. The rate of respiration was found to be nearly maximal at an oxygen partial pressure of 90 mm.Hg, the increase at higher oxygen tensions (150-760 mm.Hg) being only 6 per cent. On the other hand a decrease of oxygen tension below 90 mm.Hg caused a very marked drop in the bacterial respiration rate. At 60 mm.Hg the respiration dropped by 18 per cent, at 38 mm.Hg by 27 per cent, at 15 mm.Hg by 58 per cent and at 8.4 mm.Hg by 70 per cent. The respiratory quotient was constant at 0.75 for all oxygen tensions applied. The experiments show that the respiration of tubercle bacteria is highly dependent on variations of oxygen partial pressure, especially at those pressures actually occurring in the body.

Since the oxidative metabolism is practically the only source of energy for the tubercle bacillus, the inhibitory influence of low oxygen tension on its respiration will cause "starvation" and thereby a decrease of the injurious effects of the bacillus. This may explain the beneficial effect of various forms of treatment of tuberculosis (high altitudes, thoracoplasty, pneumothorax, phrenic paralysis), since in all these cases there is a lowering of oxygen partial pressures in the diseased tissue and consequently decreased metabolism of tubercle bacillus.

The metabolism of human myeloblasts and its sensitivity toward variations of oxygen tension. W. KEMPNER and M. GAFFRON (introduced by G. S. Eadie). Department of Medicine, Duke University, Durham, N. C. (Read by title.)

The metabolism of the blood cells of a patient with myeloblastic leukemia was measured at oxygen tensions varying from 0 to 760 mm. Hg. The blood contained 180,000 leucocytes per c.mm., 95 per cent of which were myeloblasts. Blood samples were taken in heparin and immediately shaken in the thermostat in contact with gas mixtures containing 5 per cent carbon dioxide and varying proportions of oxygen and nitrogen.

Oxygen consumption and aerobic and anaerobic glycolysis were determined manometrically by the Warburg methods.

Metabolism of 1 mgm. (dry weight) of myeloblasts per hour at various oxygen tensions
 $2.5 \cdot 10^{-2}$ M. NaHCO_3 , $8.5 \cdot 10^{-3}$ M. glucose. 37.5°C .

| MM. Hg OXYGEN TENSION | C.MM. OXYGEN CONSUMED | C.MM. CARBON DIOXIDE FORMED | RESPIRATORY QUOTIENT | C.MM. LACTIC ACID FORMED | % INHIBITION OF RESPIRATION |
|-----------------------|-----------------------|-----------------------------|----------------------|--------------------------|-----------------------------|
| 760-160 | 7.7 | 5.8 | 0.75 | 0 | 0 |
| 60 | 4.0 | 0.95 | 0.24 | 0 | 48 |
| 45 | 3.22 | 0 | 0 | 0 | 58 |
| 0 | 0 | 0 | 0 | 11.5 | 100 |

Unlike more mature myelogenous cells and tumor cells with which they frequently are compared, myeloblasts show a purely oxidative metabolism under aerobic conditions. No lactic acid formation occurs unless the cells are submitted to almost complete absence of oxygen. Myeloblast respiration is very sensitive to variations of oxygen tension, showing marked inhibition at relatively high partial pressures of oxygen. Furthermore oxidation becomes very incomplete with lowered oxygen tension: the respiratory quotient decreases gradually to zero. The fact that in young white blood cells (myeloblasts) the marked drop of the respiration rate at low oxygen tensions does *not* lead to lactic acid formation makes it seem probable that the lactic acid formation which mature blood cells, injured tissue and tumor cells show in air and even in pure oxygen is not due to inhibited respiration but rather to a direct change in their lactic acid fermentation enzyme.

*Coronary blood flow in dogs during anoxia and allied conditions.*¹ GERALD T. KENT (by invitation), R. WÉGRIA (by invitation), and HAROLD D. GREEN. Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O.

Blood flow has been recorded by the differential manometric method demonstrated at this meeting by Green and Gregg.

Asphyxia induced by stopping artificial respiration (chest opened), caused, in a typical experiment, an increase within 1.5 minutes of 290 per cent in the systolic and 90 per cent in the diastolic rate of flow with an elevation of aortic pressure of but 10 per cent. Further increase of flow accompanied the subsequent rise of aortic pressure. Control values were restored within 3 minutes after starting artificial respiration.

Artificial respiration with 5 per cent oxygen in nitrogen caused in a typical experiment within one minute maximal increases of rate of flow amounting to 85 per cent during systole and 75 per cent during diastole with only an 11 per cent increase of aortic pressure. Return to air restored the coronary circulation to control levels within 45 seconds.

Intracoronary injection of 1.0 mgm. of sodium cyanide caused within 35 seconds a maximal increase of 210 per cent in systolic and 110 per cent in diastolic rate of flow with a 5 per cent decrease of aortic pressure. The rate of flow returned to control values within 3.25 minutes. The myo-

¹ This research was aided by a grant from the Council on Pharmacy and Chemistry of the American Medical Association.

cardiographic record (Tennant and Wiggers. *Am. J. Physiol.* **112**: 351, 1935) revealed that the muscle in the infused area was not shortening during the period of maximally increased systolic flow.

The increased rate of flow caused by short periods of ischemia has been demonstrated in the paper by Green and Gregg. Longer intervals of ischemia (1+ minutes) associated with complete failure of ventricular shortening in the infused area (myocardiographic record) caused increased rate of flow, during restoration of the blood supply, amounting to approximately 100 per cent in systole and 60 per cent in diastole. Recovery was apparently complete within 3 minutes after the end of a 1.5 minute ischemia.

The relatively greater percentile increase of systolic flow in anoxia is probably associated with diminished vigor of ventricular contraction.

Studies of motor performance after ablation of postcentral areas in monkeys.

M. M. KESSLER (by invitation) and MARGARET A. KENNARD. Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn. (Read by title.)

Lesions of the parietal cortex are followed in man and monkeys by conspicuous sensory defects, but the accompanying alterations in motor performance, though often noted, have not hitherto been studied in detail. Fourteen monkeys were subjected to unilateral or bilateral ablations of: 1, the entire parietal lobe; 2, the postcentral gyrus (areas 3-1-2 of Brodmann); 3, areas 1 and 2 only; 4, the posterior parietal lobule (areas 5, 7, sometimes including 19). General behavior, neurological status and, specifically, hand performance were studied before and after operation. The effects of ablation were evident in contralateral extremities only. Bilateral ablations did not cause obvious increase in deficit in ipsilateral extremities. A static postural defect was present consisting of a limp, dangling upper extremity and a failure to flex the lower extremity completely. During progression high-stepping and circumduction appeared. After maximal bilateral lesions the limbs were held in flexion while body weight was being supported and there was uncertainty, tremor and weakness. Immediately after operation, the animals assumed a hand preference for the extremity ipsilateral to the side of the latest ablation and in the chronic state, if the lesion was unequal on the two sides, hand preference was evident on the side opposite the lesser lesion. Muscle "tone" was reduced and deep reflexes were diminished.

Hand performance was most altered. After maximal parietal ablation food was obtained by the affected extremity only by means of wild grabs which were rarely successful. After lesser postcentral lesions (areas 1 and 2) the animal used this method, but was also able to procure food by adduction of thumb against fingers. Fine apposition of thumb and index finger did not appear. In grooming, there was a loss of ability to manipulate nail and tip of the finger skilfully. The nail scratched too deeply or too little; the movements were exaggerated and were carried out from the elbow rather than by delicate flexion of phalangeal joints. It was noticeable that in all movements the animals scrutinized their performance closely, but blindfolding did not obviously alter the characteristics of the motor deficit.

The effects of lack of oxygen, and of low oxygen tensions, on the activities of Protozoa. J. A. KITCHING (introduced by A. K. Parpart). Department of Biology, Princeton University, Princeton, N. J.

An attempt has been made to investigate indirectly the respiration of various Protozoa by observation of the activity of cell processes during subjection of the organisms to low oxygen tensions, or lack of oxygen, or respiratory poisons. The Protozoa were mounted in a thin hanging drop, and hydrogen purified over hot platinized asbestos, or known mixtures of purified hydrogen and of oxygen, were supplied through flow meters.

The peritrich ciliate *Cothurnia* ceased all activity of cilia and contractile vacuole at oxygen tensions below between 1 and 2 mm. Hg. It was able to exist for limited periods under virtually complete lack of oxygen, and recovered activity almost immediately on admission of a sufficient oxygen supply. Stoppage of the contractile vacuole was followed by a swelling of the body, and sometimes by blistering, whereas recovery of the vacuole was followed by shrinkage. The effects of very dilute cyanide were similar to those of oxygen lack, and were also reversible.

Paramecium was found to retain activity down to much lower tensions of oxygen—down to small fractions of 1 mm. Hg. In virtually completely anaerobic conditions its ciliary and vacuolar activity slowed down to zero in about half an hour, and the organism finally blistered and cytolized. If blistering had only just begun the addition of a small quantity of oxygen produced recovery. *Paramecium* was found to be very insensitive to cyanide.

The spectrophotometric differentiation of keto androgens by the Zimmermann reaction. CHARLES D. KOCHAKIAN and LUVILLE A. STEADMAN (introduced by John R. Murlin). University of Rochester, Rochester, N. Y.

The Zimmermann reaction gives with all 17-keto androgens a characteristic violet color. On the other hand, testosterone, a 3-keto α, β unsaturated compound gives a brownish yellow color and androstan-3-on, 17-ol, a saturated 3-keto compound, give a violet color if the time of reaction is 10 minutes and a brownish violet if the color is developed for the usual 1 hour period. Androstenedione, which contains both the 17-keto and the 3-keto α, β unsaturated groups gives a violet pink color. These differences in the reaction of the various types of ketonic androgens give characteristically different spectrophotometric curves as determined by an accurate photographic method. The 17-keto androgens e.g. dehydroandrosterone give a sharp maximum at 5020 Å, testosterone at 5800 Å, androstenedione a broad band at 5020 to 5600 Å and androstan-3-on, 17-ol at 5600 Å if the color is developed for 10 minutes and a broad band of 5000-5600 Å if the color is developed for 1 hour.

Urine androgen extracts contain an inactive substance(s) which gives a brown-red color with the Zimmermann reaction. This impurity will completely mask the violet color of the androgens both visually and spectrophotometrically. The presence of the androgen may be detected, however, if the extract containing the androgen is run spectrophotometrically against a similar extract containing no androgen.

Blood V-factor in the normal and black tongue dog. HENRY I. KOHN and W. J. DANN (introduced by F. G. Hall). Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, N. C.

The concentration of blood V-factor (coenzymes one and two, possibly plus related unknown substances) averages about 50 per cent higher in the dog than in the normal human adult, when determined according to Kohn (Biochem. J. **32**: 2075, 1938). The blood level of the dog shows no significant change during an attack of black tongue and during the recovery period following the hypodermic administration of nicotinamide (2 mgm. per kilo per day for 5 days). In arbitrary units, the mean for each of 10 dogs is: 5.1, 5.2, 5.8, 5.9*, 6.6*, 6.8*, 6.9*, 7.1*, 7.1*, 8.0; the mean of the total of 55 determinations is 6.3. Asterisks indicate dogs which experienced an attack of black tongue.

The influence of temperature and certain hormones upon the toxicity of some hypnotics in cold and warm blooded animals. RICHARD KOHN-RICHARDS. Department of Pharmacology, Abbott Laboratories, North Chicago, Ill.

Frogs were exposed to various temperatures between 10 and 35°C. for one hour before the injection of different doses of sodium pentobarbital (Nembutal), Pentothal and paraldehyde, and then kept at this temperature for 4 to 5 hours. Dose mortality curves were constructed and the l.d. 50 at different temperatures compared. These curves became somewhat steeper with the increase in temperature. An attempt was made to calculate a "temperature-toxicity factor" using the formula for the temperature coefficient $Q_{10} = 10^{\frac{10[\lg k_2 - \lg k_1]}{[t_2 - t_1]}}$ for any temperature interval.

This factor was constant for Pentothal between 20 and 35°C., and showed a slightly increasing tendency for Nembutal and paraldehyde between 10 and 30°C. For all three hypnotics the factor was 1.41-1.60 for the 20 to 30°C. interval.

Significantly, the Pentothal and Nembutal series showed that these drugs were more toxic at 10° than at 20°C. This finding was checked by identical results in two winter seasons. No satisfactory explanation can be given at the present. However, the decrease of the detoxification rate and increase of the depressant action of the drugs upon the circulation may play a part. Paraldehyde did not show this phenomenon. At 20°C. thyroxine treated frogs under Nembutal showed a curve approximating that of untreated animals under Nembutal at 30°C., presenting a definitely increased sensitivity with the enhanced metabolism.

Similar experiments in mice with Pentothal demonstrated the increase of toxicity from 24°C., where the drug produced a marked drop of the body temperature, to a temperature of 37°C., where no change in body temperature occurred as the animals were kept in an incubator. The calculated temperature toxicity factor was 1.26. Adrenalectomized mice, used 2-3 days after the operation, showed a markedly lowered resistance against increase of the temperature with and without the administration of hypnotics.

Blood and blood pressure changes following carbon arc irradiation. I. A micro method for the estimation of histamine in blood. HENRY VON KOL-

NITZ (introduced by Henry Laurens) Laboratory of Physiology, Tulane University School of Medicine, New Orleans, La. (Read by title.)

Histamine is determined in volumes of 0.2-0.5 cc. of dog's blood. Microchemical technique using small volumes of reagents and a simmering water bath make the trichloroacetic acid extraction simpler and more rapid than formerly. A Katz micro bio-assay bath (J. Pharmacol. and Exper. Therap., **64**: 314, 1938) is used for the titration and, with guinea pig ileum, volumes of 0.1-0.5 cc. of a concentration of 0.0025 γ /cc. of histamine base give reproducible curves. Histamine added to blood is quantitatively recovered.

The method is being used to follow the changes in blood histamine (or a substance similar in nature) which occur when normal and hypertensive dogs (Goldblatt) are irradiated with a carbon arc. Preliminary observations, employing systemic rather than skin blood samples, have shown increases in histamine correlated simultaneously with falls in blood pressure, e.g. blood pressure 196/160 mm. Hg and blood histamine 0.025 γ /cc. before, and blood pressure 160/100 mm. Hg and blood histamine 0.115 γ /cc. after treatment.

The work is being aided by a grant from the Committee on Scientific Research of the American Medical Association.

Further studies on gastrin. S. A. KOMAROV. Department of Physiology, McGill University, Montreal, Canada.

A perfected method for the isolation of "gastrin" from dog's and hog's pyloric mucosa has been developed. The method ensures a good yield (from 1 to 1.2 per cent) of the preparations, 15 to 20 mgm. of which, when injected intravenously, elicit 1 cc. of gastric secretion in acute experiments on cats under chloralose-urethane anesthesia. The preparations were found to be devoid of vasodepressor substances but were usually slightly toxic, affecting motor and respiratory centers of the brain. Simple methods for further purification were also devised, whereby the activity of the preparations could be increased about ten times and the toxic effects entirely eliminated. The same chemical procedures, when applied to fundic mucosa or to liver, resulted in preparations devoid of gastric excitants. Experiments carried out on normal dogs with a gastric fistula and esophagotomy demonstrated that gastrin preparations elicit a gastric secretion characterized by high acidity and low pepsin content not only on intravenous but also on intramuscular administration.

Some effects of brief experimental hyperthyroidism on the rat ovary. C. P. KRAATZ (introduced by L. B. Nice). Department of Physiology, Chicago Medical School, Chicago, Ill.

Brief hyperthyroidism in adult female rats has been found to increase the litter size when the period of treatment precedes mating (Kraatz. Proc. Soc. Exper. Biol. and Med. **40**: 499, 1939). Attempts to analyze this phenomenon have led to experiments on the immature female rat.

Gonadogen (Upjohn) was injected once daily subcutaneously for a 3-day period and thyroid substance was fed by medicine dropper once daily for a 3-day period using 21-23 day old female rats. The ovaries and drained uteri were weighed 48 hours after the last injection. The findings may be summarized as follows:

1. Thyroid in 0.1 gram doses had no macroscopic effect.

2. Simultaneous administration of Gonadogen in $\frac{1}{2}$ unit doses and thyroid in 0.1 gram doses evoked differing effects in purely follicular and in luteal ovaries. Hyperthyroidism inhibited the growth response of the follicular ovaries, while the uteri of these hyperthyroid rats were much larger than those of rats receiving Gonadogen alone. On the other hand, when luteinization had taken place, the ovaries of the hyperthyroid rats were heavier than those of the Gonadogen controls.

3. With Gonadogen in $\frac{1}{3}$ unit doses, the same inhibition of growth of follicular ovaries was observed in hyperthyroid rats, while the augmentation of uterine weight was not as marked as with the smaller dosage. Luteinization was infrequent in this series.

4. When the period of thyroid-feeding (0.1 gram doses) began 3 days before the period of injections, the hyperthyroid animals responded with increased ovarian weights in both the $\frac{1}{2}$ unit series (largely luteal) and the $\frac{1}{3}$ unit series (mainly follicular), while the uteri were reduced in size compared to the respective Gonadogen controls.

5. The administration of thyroid in 0.3 gram doses appeared to be inhibitory to ovarian growth even when the treatment preceded injections. The uterine effects were similar to those observed with the 0.1 gram dosage.

The function of the musculature at the duodenal end of the pancreatic ducts in man. BERNARD L. KREILKAMP (by invitation) and EDWARD A. BOYDEN. Department of Anatomy, University of Minnesota, Minneapolis. (Read by title.)

In 1899 Helly described longitudinal and circular muscle around the accessory pancreatic ducts of human fetuses (the so-called Helly's sphincter). Subsequently Anrep (1914) showed that subdiaphragmatic stimulation of the vagus inhibited the pancreatic flow in dogs by constricting the muscle of the intrapancreatic ducts, and just recently Auer and Seager have reported that injection of secretin into rabbits causes the ampulla of the duct of Santorini to undergo movements similar to those induced in the biliary ampulla by ingestion of food (Proc. Soc. Exper. Biol. and Med. 39: 542, 1938).

Accordingly, it has seemed desirable to analyze the musculature at the duodenal end of the human pancreatic ducts. This has been accomplished by making wax models of the pancreatico-duodenal junction (in fetuses) and macerated preparations (in adults).

The conclusions of this study are that the terminal sheath of smooth muscle that invests the accessory duct arises in situ by differentiation of mesenchyme and not through emanation from the intestinal muscle, as postulated by Porsio (1933). In general the longitudinal bundles predominate, most of the fibres being so arranged as to attach the accessory duct to its window or to shorten and erect the minor papilla, thus facilitating the discharge of pancreatic juice. In about half the adult specimens variable arrangements of circular and oblique fibres occur around the base of the minor papilla. These never constitute a continuous sheath of sphincter fibres, such as occurs along the intramural course of the common bile duct, nor is the arrangement such as to favor peristalsis.

Maceration preparations of the major papilla reveal the existence of a sphincter pancreaticus above the ampulla of Vater in only 4 out of 12 preparations. Two of the four showed a well developed sphincter ampullae.

Taken as a whole, therefore, the musculature of the two pancreatic ducts seems designed to erect the papillae and to tie the ducts to their respective windows; only in a minority of cases are sphincter fibres sufficiently developed to offer resistance, and then much less effectively than the corresponding sheath that envelopes the end of the common bile duct.

Rhythmical variation of respiratory excursion associated with bilateral injury of the efferent fibers from the cerebral cortex. ORTHELLO R. LANGWORTHY, RUDOLF V. GRIMMER (by invitation) and FREDERICK H. HESSER (by invitation). Johns Hopkins University, Baltimore, Md.

Patients with injury of the efferent fibers from the cerebral motor cortex bilaterally show a rhythmical variation of respiratory excursions. This is similar to Cheyne-Stokes respiration, especially in the more marked cases. These patients were not unconscious nor did they have manifest circulatory inadequacy. Indeed the respiratory changes persisted practically unaltered for weeks or months. Associated with injury of the corticospinal and corticobulbar fibers on both sides they showed paresis of the extremities, difficulties in speaking and swallowing, and emotional instability. There was little ability to increase the amplitude of the respiratory excursions voluntarily. The administration of coramine, aminophyllin, oxygen or carbon dioxide tended to decrease the periodicity. They produced the same effect as in other types of Cheyne-Stokes respiration. Hughlings Jackson suggested that the respiratory center in the medulla is released from normal control of both cerebral cortices. Under these conditions it tends to react excessively to stimuli.

A study of the manner of combination of the central and reflexogenic chemical drives of the act of breathing. JACK LAPIDES (by invitation), MANUEL LEVIN (by invitation) and ROBERT GESELL. Department of Physiology, University of Michigan, Ann Arbor.

Temporary cold-blocking of Hering's nerves produced an increase, decrease, or no change in respiration depending upon the individual animal. Using vagotomized dogs, similar effects were obtained with and without a blood-pressure compensator. Deflating the carotid sinuses without destroying the carotid bodies converted a preceding increase in respiration upon blocking to a decrease or no change.

Cold-blocking during anoxic hyperpnea produced a depression of respiration reverting to hyperpnea on deblocking. This depression increased with the decrease in oxygen in the range of 21 per cent to 8 per cent. With low percentages of oxygen, after long apneas produced by blocking Hering's nerves, a slow gasping respiration developed which was unaffected by blocking. This indicated apparently a new central stimulation developing after the reflex mechanism became paralyzed.

In animals showing a tonic carotid body activity, the effect of blocking was gradually reduced during increasing hypercapnic hyperpnea with increasing percentages of CO_2 and was eliminated with approximately 5 per cent CO_2 .

Hyperpnea produced by cyanide injections was changed to subnormal respiration when the Hering nerves were blocked and deblocking restored hyperpnea. This is similar to the effect noted with low oxygen.

Apapnic apnea following artificial hyperventilation was affected by cold-blocking Hering's nerves. When a block was applied just before stopping

artificial ventilation, the ensuing apnea was longer in 50 per cent of the experiments and recovery of normal respiration was delayed in all.

Hemorrhaging the vagotomized dog with sinuses deflated produced a stimulation of respiratory rate. There was also a transitory increase in amplitude soon changing to a decrease so that respiratory volume was decreased. With Hering's nerves blocked, the effect was similar but the increase in rate was less and the depression of amplitude more, resulting in a still smaller respiratory volume.

The effect of chemical agents on the excitability of ganglion cells. M. G. LARRABEE (by invitation), F. BRINK (by invitation) and D. W. BRONK. Johnson Foundation, University of Pennsylvania, Philadelphia.

The number of ganglion cells responding to a submaximal preganglionic volley is modified by perfusing the ganglion with solutions which are known to alter the electrical excitability of peripheral nerve. We have studied these effects on synaptic transmission by stimulating the preganglionic nerve to the cat's stellate ganglion and recording the postganglionic action potentials.

A tenfold increase in potassium concentration produces an initial increase in the number of ganglion cells responding to a preganglionic volley, followed by block of synaptic transmission. An equal increase of calcium concentration at first depresses and finally blocks transmission. These observations agree with those of Brown and Feldberg. Neither the block produced by high potassium nor that by high calcium need be considered a specific action on the synaptic mechanism. For we have observed that the same potassium concentration abolishes transmission over axons which pass directly through the ganglion. On the other hand the concentration of calcium employed does not block these direct fibers. However, since high calcium is known to raise the electrical threshold of peripheral nerve, it may prevent synaptic transmission by a similar elevation of threshold of the ganglion cell.

Complete removal of potassium occasionally produces a small increase in the number of ganglion cells responding to a preganglionic volley, but generally it has no observed effect on the ganglionic mechanisms. This is in contrast with low calcium which causes a discharge of rhythmically recurring impulses from the ganglion cells. These effects again are in agreement with the properties of peripheral nerve.

While the cells are discharging rhythmically due to low calcium, they cannot be excited by presynaptic impulses. This appears to be a specific interference with synaptic transmission, for impulses are still conducted by fibers which pass directly through the ganglion.

Excepting the block produced by low calcium, we have thus found that changes in certain ionic concentrations produce effects on ganglionic transmission which can be explained by assuming that they are due to changes in the irritability of the ganglion cells. The changes are analogous to the alterations of electrical excitability known to be produced by these ions.

The influence of the hypophysis in gonadotropic assays. HENRY D. LAUSON (introduced by W. J. Meek). Departments of Medicine and Physiology, University of Wisconsin Medical School, Madison.

Gonadotropic assays have been conducted on a large number of hypophyses taken from immature female rats which had been employed as

test animals in other gonadotropic assays. The pituitary gonadotropic content was found to be inversely proportional to the degree of genital development. That is, hypophyses of rats, the ovaries of which were considerably enlarged, contained less than hypophyses from rats in which gonad-stimulation was less marked. The maximum decrease was 40 per cent below normal.

In a definitive experiment, 48 rats were divided into four groups of 12 each. Two groups were ovariectomized on the 21st day of life while the other two groups were left intact. Beginning on the 22nd day of life, one normal and one castrated group were injected with a rat pituitary gonadotropic preparation twice daily for three days. Mean ovary weight of the injected normals was 38.9 mgm., range 24.6 to 66.8 mgm. All hypophyses from the four groups were assayed individually on 48 twenty-two day old female rats. Each test rat received a total of $\frac{3}{4}$ of one pituitary in 6 doses. The mean uterine (and ovarian) weights from the test rats were as follows: 85.4 mgm. (20.4 mgm.), 44.2 mgm. (12.3 mgm.), 78.6 mgm. (18.0 mgm.), 83.5 mgm. (17.0 mgm.) for the normal control, normal injected, castrate control, castrate injected, respectively. These results, considered in the light of related experiments in the literature, indicate that ovaries stimulated by exogenous gonadotropic hormone cause the hypophysis to release endogenous hormone, which, in turn, augments the ovary-stimulating action of the injected material. This endogenous contribution is proportional to the exogenous dose, up to a certain limit.

The influence of intestinal ischemia on the absorption of histamine. HAMPDEN LAWSON and R. F. ANTONCIC (by invitation). Department of Physiology, University of Louisville School of Medicine, Louisville, Ky.

In barbitalized dogs histamine dihydrochloride in doses of 50 to 100 mgm. injected into small closed loops of the jejunum, ileum or colon was without effect on blood pressure or respiration. If blood flow through loops containing these doses of histamine was stopped for as long as ten minutes by clamping the artery and vein, an abrupt fall in blood pressure occurred on renewing the circulation. Blood pressure usually began to return toward normal within one minute of re-application of the clamp to the blood vessels. Unless this was done pressure continued to fall to the death of the animal. Control observations without histamine injection were made on loops into which equal or larger volumes of 0.9 per cent NaCl had been injected. Renewal of the circulation through such control loops after periods of ischemia lasting as long as three hours was without effect on blood pressure.

If untreated loops were kept ischemic for thirty minutes or longer, injection of histamine during the post-ischemic period caused a drop in blood pressure. In one experiment histamine injected ninety minutes after renewal of the circulation in a loop which had been without blood flow for three hours caused a fatal drop in blood pressure. In loops subjected to shorter periods of ischemia, however (up to fifteen minutes), histamine injected even during the second or third minute of recovery was without effect.

Observations on six animals surviving experimental obstruction of the jejunum for 24-48 hours failed to show a significant difference in the toxicity of histamine injected above and below the obstruction.

These data are taken to mean an increased entrance of histamine into

blood from intestine damaged by ischemia. Of the two possible mechanisms, accelerated histamine uptake from the gut, and retarded histamine detoxication, the latter seems the more likely. The data offer no evidence that intestinal obstruction produces similar changes.

The prognostic significance of the S-T interval in hypertension. BERTRAM LEVINSON (introduced by Jane Sands Robb). Departments of Medicine and Pharmacology, College of Medicine, Syracuse University, Syracuse, N. Y.

The electrocardiogram of hypertension is well known. Depending on the amount of cardiac strain and damage a variable degree of left axis deviation, negativity of T_1 and T_2 , some depression of $S-T_1$ and T_2 , and sometimes elevation of T_3 and $S-T_3$ are present. There may also appear a widened QRS, prolonged QT, increased voltage in leads 1 and 3 and a low notched R_2 and S_2 . The prognosis is considered to be more serious in discordant laevograms (Luten and Grove, Freundlich), and where S-T cannot be returned to normal by Amyl Nitrite (Nagl). In our 100 cases there were far fewer S-T shifts of more than 1 mm. than were found in the records of normal students. Most of the shifts were negative and many had negative T waves in leads 1 or 2 or both. A correlation apparently exists between the direction of the S-T displacement and the direction of the T wave. In the student records all T waves were positive and most S-T displacements were upward while the reverse is true of hypertensives. Such S-T displacements could be produced if the T wave began before the end of S. It may be that prognosis is related to the direction and amplitude of T rather than to any displacement of S-T.

*Further studies on the refractory state developed following repeated injections of adrenal extract.*¹ LENA A. LEWIS (by invitation), FRANK A. HARTMAN and JANE E. GABRIEL (by invitation). Department of Physiology, Ohio State University, Columbus.

It has been reported previously that repeated intravenous injections of adrenal extract develop a refractory state in normal subjects (Hartman, Lewis and Toby—Science **86**: 128, 1937).

The refractory state failed to develop in a normal female dog following four intravenous injections of 4 mgm. of corticosterone in 10 per cent alcohol. Four injections of standard adrenal extracts are sufficient to confer complete refractoriness. A retention of sodium of 29 per cent was given on the initial injection and of 27 per cent on the final injection of corticosterone.

One milligram of desoxycorticosterone in oil subcutaneously caused a retention of sodium equivalent to 6 mgm. of corticosterone.

All of the earlier refractory studies have been carried out on normal animals. They have now been extended to include adrenalectomized dogs. Three adrenalectomized dogs repeatedly injected intravenously at intervals of three to four days with 40 cat units of adrenal extract twice daily developed the refractory state and failed to show any increased sodium retention on further injection. The plasma electrolyte picture of adrenalectomized dogs thus made refractory was essentially that of adrenalectomized animals carried on cortin alone (Hartman, Spoor and Lewis—Science

¹ Aided by a grant from The Rockefeller Foundation.

—in press). Two dogs thus made refractory showed the usual increased sodium retention following injection of 1.5 mgm. desoxycorticosterone.

It has been reported that the refractory state could be conferred on a previously uninjected dog by the intravenous injection of 20 cc. dog serum from a refractory dog. It has now been shown that the pseudoglobulin fraction of the serum from refractory dogs is also capable of producing refractoriness in normal untreated dogs.

The quantitative relation of reactive hyperemia in the heart to the duration of ischemia. ERNA LINDNER (by invitation) and LOUIS N. KATZ. Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

Eicholtz and Hilton (1) have reported that anoxemia in the heart-lung preparation leads to coronary dilatation. We (2) have demonstrated that this occurs in ischemia of the isolated heart. To study the quantitative aspects of this dilatation, we used an isolated dog-heart preparation with ventricles fibrillating, in which the coronaries were perfused with defibrinated blood at constant temperature and pressure. Total coronary outflow was used as the index of coronary calibre. Ischemic periods of varying duration were produced by abrupt interruption of coronary inflow. The following results were obtained: 1, the total excess flow, disproportionately, was considerably less than the interrupted flow; 2, there was little correspondence between the duration or amount of hyperemia and the length of the ischemic period; however, 3, the percentage increase in flow, and especially that for the first two minutes after ischemia, was definitely related to the duration of the latter.

Like other regions of the body, the heart has a mechanism, independent of nerves or humoral agents, which can make up almost quantitatively the debt incurred during ischemia. This mechanism is metabolic in origin but conclusive information is lacking regarding the exact nature of the responsible substances. The lack of a quantitative relationship between the total duration or amount of reactive hyperemia and the ischemic period suggests that the metabolic substance(s) responsible for the coronary dilatation is diffusible and is washed away by the renewed inflow of blood. The lack of correlation of the reactive hyperemia to the magnitude of the interrupted flow suggests that the coronary flow is in excess of myocardial needs.

Reactive hyperemia tends to nullify the deleterious effects resulting whenever the coronary blood flow becomes insufficient to maintain a normal state in the beating heart. Since cardiac activity is incessant, this mechanism permits restoration of the requirements of the heart after any temporary or relative coronary ischemia. Obviously, the responsiveness of the coronary arteries determines the efficacy of compensation. In addition, a limit exists beyond which the degree of ischemia cannot be counteracted; at this point either failure of the heart or necrosis of the local region ensues.

(1) J. Physiol. **59**: 413, 1925.

(2) Am. J. Physiol. **122**: 252, 1938.

Liberation of chemical mediators by stimulating isolated nerves. K. LISSÁK (introduced by W. B. Cannon). Harvard Medical School, Boston, Mass.

Isolated nerves of cats and frogs (sciatic only) were stimulated in vitro

in Ringer-solution containing physostigmine (1:100,000) and the solution then tested on the isolated frog heart. Parasympathetic, motor and preganglionic nerves and vagal and sympathetic ganglia liberated acetylcholine in different amounts (cf. Binet and Minz, 1934). If only the cut ends of the nerve were submerged in the solution and the mid portion stimulated the acetylcholine liberated was about twice as much as when the mid portion was submerged and one end stimulated. If the cut ends were in the solution for 20 minutes even without stimulation some acetylcholine appeared. Stimulation of the isolated, degenerated sciatic did not liberate acetylcholine. Contrary to observations by Minz (1938) on leech muscle, stimulating the vagus or other cholinergic nerves (sciatic) did not liberate a substance which sensitizes the frog heart to acetylcholine; also treatment of the frog heart with vitamin B₁ or B₂ and with extract of yeast produced no sensitization to acetylcholine.

Stimulation of postganglionic sympathetic fibers and mixed nerves (sciatic) which contain adrenergic fibers, in Ringer-solution without physostigmine, liberated in different amounts a substance which has an adrenaline-like action on the hypodynamic frog heart.

Usually when any isolated nerves are stimulated there appear, besides the chemical mediators, some lipoids and proteins. If the solution is tested for acetylcholine on the maximally beating normal frog heart, the amounts of lipoids and proteins are without influence. If solutions are tested for adrenaline on a hypodynamic frog heart, however, these have a strong positive action and therefore confuse an adrenaline-like effect. The large molecules can be excluded, however, by dialysis. When adrenergic fibers are stimulated in Ringer-solution and the solution dialyzed, the dialyzate has adrenaline-like positive inotropic and chronotropic effects on the hypodynamic frog heart. These effects are abolished by ergotoxine, or by previously heating or by ashing or oxidizing the solution. The substance liberated when adrenergic fibers are stimulated in vitro has properties similar to adrenaline.

BINET, L. AND B. MINZ. *Compt. Rend. Soc. Biol.* **116**: 107, 1934.

MINZ, B. *Ibid.* **127**: 1251, 1938.

The excitability cycle of inferior mesenteric ganglion cells following antidromic activation. D. P. C. LLOYD (introduced by V. E. Henderson). Department of Medical Research, University of Toronto, Canada.

Supernormality and subnormality, being threshold alterations, must exist at the point of excitation to influence the response to that excitation. Since synaptic excitation of the ganglion cell must occur in the region of the synapses, then supernormality and subnormality revealed by synaptic stimulation of the ganglion cell (obtained by maximal stimulation of a fraction of the preganglionic inflow) are a function of the cell body and dendrites. Synaptic stimulation tests the cellular excitability only within the fixed range determined by density of active preganglionic endings.

The excitability cycle of inferior mesenteric ganglion M₂ (S₂) cells, tested by a preganglionic volley, following an antidromic conditioning volley shows distinct supernormal and subnormal periods. The early course of the excitability cycle is significantly lower than that following preganglionic conditioning (Lloyd. *Am. J. Physiol.* **123**: 129, 1938) although the time course is similar. This discrepancy is not due to recruitment

from subliminal fringe following preganglionic conditioning as this has been shown not to occur in the M_2 cell group of the I. M. G.

Since the qualitative agreement is good between the excitability cycles following preganglionic and antidromic activation, and since the region excited by the testing synaptic stimulation is the same in both cases, they must represent fundamentally the same cellular process. The quantitative disparity, however, suggests the differential action of extracellular influences (possibly liberated acetylcholine) or of incidental factors such as the direction in which the conducted disturbance sweeps over the cell and dendrites, as these regions of the neurone lack the trans-sectional uniformity of the axone.

In one experiment of the present series the supernormal and subnormal periods were not definable after either preganglionic or antidromic conditioning, the test responses being uniform with controls throughout. As has been demonstrated previously in the case of preganglionic conditioning alone (loc. cit.) this indicates that the synaptic stimulus to all the responding cells was sufficiently supraliminal to overcome the raised threshold of subnormality following antidromic conditioning as well.

The effect of duodenal instillation of hydrochloric acid upon the fasting blood-sugar of dogs. E. R. LOEW¹ (by invitation) and J. S. GRAY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The hypoglycemic response purported to occur following the injection of duodenal extracts or duodenal instillation of hydrochloric acid has suggested that a duodenal hormone is concerned in the regulation of carbohydrate metabolism. The introduction of 100 cc. of 0.3 per cent hydrochloric acid into the intestine of gastrectomized dogs with jejuno-esophageal anastomoses and total pouch dogs with duodeno-esophageal anastomoses, or into the stomach of intact animals has failed to reveal a reduction in fasting blood-sugar levels over a five-hour period (18 experiments). Furthermore, duodenal instillation of dilute acid in 12 dogs or saline in 8 dogs under pentobarbital (nembutal) anesthesia caused no appreciable alteration in the average blood-sugar concentration.

Similar experiments were performed using nembutalized, adrenal-inactivated dogs in an attempt to confirm the published reports that such animals exhibit hypoglycemia following acid stimulation of the duodenum. The average blood-sugar concentration was increased shortly after ligation of the adrenal veins, then decreased during a two-hour control period before acid or saline was introduced into the duodenum. This progressive decrease in blood-sugar was not significantly accelerated by the introduction of 50 cc. of 0.3 per cent hydrochloric acid into the duodenum of 8 dogs, for a decrease of the same magnitude occurred in the 8 saline-control dogs. Following bilateral extirpation of the adrenal glands in 7 dogs the decrease in blood sugar was even more pronounced before and after saline was placed in the duodenum. The data secured from adequately controlled experiments upon adrenal-inactivated dogs refute the contention that duodenal stimulation with acid effects a hypoglycemia and also emphasize the fact that some of the data previously published have been secured under unphysiological and improperly controlled conditions.

¹ Porter Fellow in Physiology, 1938-39.

Our results obtained from 64 experiments upon 55 dogs have failed to yield evidence which suggests that acid stimulation of the duodenum liberates a hormone which regulates carbohydrate metabolism, but do not disprove the existence of such a mechanism.

Some biophysical relations of devitrification temperatures. B. J. LUYET.
Department of Biology, Saint Louis University, Mo.

When a colloid or protoplasm, of convenient water-content, is immersed in thin layers in liquid air ($-190^{\circ}\text{C}.$) it vitrifies. On warming, the vitreous material crystallizes at temperatures called devitrification temperatures. The latter are somewhat comparable, as physical characteristics of a substance, to the melting or the boiling point. The devitrification temperatures can furnish some information on: *a*, the order of magnitude of the molecular weight; *b*, the amount of bound water; *c*, the cause of protoplasmic death by low temperature and of survival in the vitreous state.—*a*. The existence of a relation between the devitrification temperatures and the structure and mass of the molecules is shown by the following comparative values for solutions of sugars: Glucose 2M (6 carbons): -40.6° ; Sucrose 2M (12 carbons): -31.8° ; Sucrose 1M: -31.4° ; Raffinose 1M (18 carbons): -27.2° ; Dextrin 2M/ χ (6 χ carbons): -9.4° ; Dextrin 1M/ χ : -9.2° ; (standard devitrification time: 5 minutes).—*b*. A saturated solution of sucrose does not devitrify at any temperature. When the solution is diluted to 9 or 10 molecules of water for 1 molecule of sucrose it begins to devitrify slowly when exposed to -45° . When the number of molecules of water increases to 13 or 14 there is a transition to another type of crystallization and the devitrification temperatures are displaced upward by some 15 degrees. Some 10 molecules of water, therefore, are bound to sucrose in such a way that they never freeze, 3 or 4 molecules induce one type of freezing, and the large bulk of excess molecules in more diluted solutions crystallize freely. *c*. Isolated frog's muscle fibers respond to an electric stimulus after having been vitrified in liquid air if they are warmed up rapidly by sudden immersion in warm saline, while, if they have been left for a few minutes in the neighborhood of -30° after vitrification, a high percentage of them cease to respond. The devitrification temperature of the extracted myoplasma being about -29° , it is suggested that devitrification at that temperature is the cause of death.

The effect of sulfanilamide on the acid-base balance of dogs. E. W. MCCHESENEY (by invitation), K. D. SPRAGUE (by invitation), and O. W. BARLOW.
Winthrop Chemical Company, Inc., Rensselaer, N. Y.

When dogs are given large doses of sulfanilamide by mouth (500 mgm. per kgm. per day, in three equal doses), blood sulfanilamide rises to about 50 mg. per cent and most of the drug (75 per cent or more) can be recovered unchanged in the urine. The plasma CO_2 combining power gradually decreases from 50-55 to 40-45 volumes per cent. The normal level may be maintained (or is rapidly restored) by giving, simultaneously with the drug, 200 mgm. per kgm. of magnesium oxide, or 750 mgm. per kgm. sodium bicarbonate per day.

The administration of the above-mentioned quantity of sulfanilamide as a single dose, daily for two days, causes: 1, a slight rise in blood pH noted after 5 hours; 2, a simultaneous marked rise in urine pH and CO_2 , and 3, a gradual loss in plasma CO_2 . This has been observed previously

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by Marshall, Cutting, and Emerson (J. A. M. A. **110**: 255, 1938) on dogs, and in part by numerous investigators on humans. These phenomena have been taken by some to indicate that the change in acid-base balance induced by sulfanilamide is of the primary CO_2 deficit type. However, our own data (based on blood, urine, and respiratory changes and their exact sequence) indicate that the disturbance begins with an alkali loss through the kidneys (primary alkali deficit).

When sulfanilamide is given in two doses daily (250 mgm. per kgm. each) for a number of days, a systematic study of the acid-base balance fails to reveal any significant change except in the CO_2 -combining power.

The administration of an equivalent quantity of acetylsulfanilamide (single dose of 620 mgm. per kgm. per day for 2 days) causes: 1, a marked drop in blood pH on the second day; 2, a rapid fall in plasma CO_2 , particularly on the first day, and 3, an increase of urinary pH and CO_2 on the first day, continuing at slightly elevated levels through the second. The dogs do not appear to be made very ill by the drug. Excretion data show that a rather large portion of the drug (25 per cent or more) escapes absorption; the blood level does not rise above 4 or 5 mgm. per cent.

Antiketogenic and glycogenic activity of citric acid and the ketogenic activity of acetic acid and sodium bicarbonate. EATON M. MACKAY, HERBERT O. CARNE (by invitation) and ARNE N. WICK (by invitation). The Scripps Metabolic Clinic, La Jolla, Calif.

Rats fed a diet low in protein and choline develop fatty livers and when fasted a significant ketosis occurs. This ketosis probably results from the low protein intake rather than the fatty liver, for preliminary experiments indicate that the addition of choline to the diet prevents the deposition of fat in the liver but has no effect upon the ketosis. This ketosis provides a good medium for study of antiketogenesis. Usually the ketonuria follows the ketonemia in which case it is a satisfactory measure of the ketosis. Under some conditions the blood ketones must be determined.

Citric acid in suitable quantities had a marked antiketogenic action, measured by both the ketonemia and ketonuria. Sodium bicarbonate given to such rats increases the ketonuria due to the alkalosis. Under such conditions citric acid has no antiketogenic activity. A sodium bicarbonate alkalosis causes citric acid synthesis in the dog (Sherman, Mendel, Smith and Toothill. J. Biol. Chem. **113**: 247, 1936) and it is possible that citric acid may not be utilized in the presence of an alkalosis.

Like other antiketogenic agents citric acid may form glycogen. Fasting rats receiving citric acid utilize less protein as measured by urine nitrogen, no more fat, and have significantly higher liver and extrahepatic glycogen concentrations than comparable fasting rats given no citric acid.

Acetic acid fed to fasting rats with a ketosis was ketogenic. Excretion of ketones and nitrogen in the urine were increased. Acetic acid is known (Lusk, J. Biol. Chem. **49**: 453, 1921) to stimulate metabolism. The extra ketone bodies probably originated from the acid rather than the increased protein catabolism or total metabolism for acetic acid gives rise to acetoacetic acid in the perfused liver and in liver slices.

The reason for the ketogenic action of sodium bicarbonate is still not clear. In the fasting rat this alkali reduces protein catabolism to some extent and this may be responsible. Since bicarbonate reduces the use of protein and also spares the body fat of the fasting rat it must cause a reduction in the total metabolism which has been overlooked.

Cord potentials in spinal shock: contralateral effects. G. P. McCouch, JOSEPH HUGHES and WINIFRED B. STEWART (by invitation). Department of Physiology, University of Pennsylvania, and Pennsylvania Hospital for Mental and Nervous Diseases, Philadelphia.

In the monkey the internuncial potential is localized to a more limited area than in the cat and this restriction is inversely related to the interval between transection and recording. When recorded from the lateral part of the surface of the dorsal columns immediately above the entrance zone of the dorsal root stimulated, in the acute and subacute macaque, the internuncial potential is limited to the ipsilateral side. In the monkey in which transection has been preceded by hemisection with consequent recovery of reflexes upon the hemisected side before their return upon the acute side, the internuncial potential arises from a more extensive region on the chronic side than on the acute side. Consonant with the absence of a crossed potential is the absence of crossed reflexes either excitatory or inhibitory within the first few weeks. In the chronic monkey there is eventually recovery of crossed effects both excitatory and inhibitory. In the chronic monkey and even in the chronic dog, contralateral inhibition of the ipsilateral flexor reflex has been obtained only from stimuli involving a large proportion of B fibers of the contralateral nerve. In the cat, such strong inhibitory stimulation is effective even in the acute preparation. In the chronic spinal cat, the threshold of contralateral reflexes approximates that of ipsilateral responses for both excitatory and inhibitory components. Spinal shock depresses excitatory and inhibitory reflexes equally. It depresses crossed reflexes, more than ipsilateral reflexes. So long as crossed inhibition of reflexes is absent, there is a parallel absence of inhibition of the internuncial negativity associated with the test volley. Hence, even in the monkey, the limiting factor in shock to crossed inhibition is at the internuncial level.

The effect of sodium sulfanilyl sulfanilate upon the development of fixed virus rabies in rabbits. F. D. McCREA. Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, N. C.

Rabbits were inoculated intracerebrally with 0.1 cc. per kilogram of 1:50, 1:100 and 1:200 dilutions of rabies fixed virus obtained from rabbit spinal cords, the weakest dilution being twice the average fatal dose. One third of the animals in each group were kept as untreated controls; the remainder were given sodium sulfanilyl sulfanilate in 10 per cent solution, usually intravenously. Dosages varied from 0.5 gram per kgm. daily to 1.0 gram per kgm. twice daily for periods of 3 to 7 days. These doses administered to uninfected rabbits for 8 to 10 days produced no evident toxic effects. Experiments in which all the untreated controls did not develop rabies in 9 days were discarded. Exclusive of these 49 treated animals are reported.

Of the 49 treated animals, 28 died of rabies, 3 of upper respiratory infection and 18 survived for periods of at least 90 days after the death of the appropriate controls. Thus approximately one third of the treated animals did not develop rabies.

Because of the small number of animals involved it is difficult to make any statement about the effects of virus dilution and of dosage. Results with different virus dilutions are as follows: Using 1:50 dilutions, of 6 treated animals, one survived, 4 died of rabies and one of upper respiratory infection. Using 1:100 dilutions, of 12 treated animals, 5 survived, 5 died

of rabies and 2 of upper respiratory infections. With 1:200 dilutions of 31 treated animals 12 survived and 19 died of rabies.

When 1.0 gram per kgm. per day of the drug was used, of 27 treated rabbits, 11 lived and 13 died of rabies. With 1.0 gram per kgm. administered twice daily to 3 animals, all died of rabies. Using 0.75 gram per kgm. per day, of 8 treated animals, one lived and 7 died of rabies. With 0.75 gram per kgm. administered twice daily, of 3 treated rabbits, all died of rabies. Using 0.50 gram per kgm. per day, of 6 treated animals, 4 survived and 2 died of rabies. The same dose was administered twice daily to 2 rabbits, both survived.

It would thus seem that sodium sulfanilyl sulfanilate prevents the development of rabies in about one third of the animals treated. The experiments are being continued.

The knee-jerk following facilitating and extinguishing stimulation of related cortical foci. W. S. McCULLOCH and J. G. DUSSER DE BARENNE. Laboratory of Neurophysiology, Yale University School of Medicine, New Haven, Conn.

Changes in action potentials, afterpotentials and pH shown to be factors for facilitation and extinction at the site of stimulation and in neighboring cortical foci have been demonstrated in distant parts of the central nervous system.

The effects of these changes in remote parts of the central nervous system belonging to the system stimulated were studied by eliciting the knee-jerk periodically and stimulating a cortical focus for extension at the knee with facilitating or extinguishing stimulation. Stimulation giving primary facilitation at such a focus facilitates the knee-jerk; stimulation giving extinction at this focus extinguishes the knee-jerk. Precise thermocoagulation of this same focus promptly abolishes the knee-jerk which returns very late and small.

The effects of these changes in remote parts of the central nervous system pertaining to systems reciprocally related to the extensor systems involved in the knee-jerk were studied by stimulating a cortical focus for flexion at the knee. Extinguishing stimulation of such a focus enhances the knee-jerk; facilitating stimulation of that focus obliterates the knee-jerk. This last phenomenon is a true central inhibition in that it is one aspect of reciprocal innervation. In this it differs from extinction which does not depend upon any change in a reciprocally related system, although inhibition must ultimately depend upon factors for extinction in the system under investigation due to excitation in the reciprocally related system.

On the purification and "tachyphylaxis" to renin. E. G. McEWEN and S. P. HARRISON¹ (introduced by A. C. Ivy). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

In the course of studies on the purification of various fractions of kidney extracts the appearance of "tolerance" to equal successive doses of active preparations injected at equal intervals into blood pressure assay animals was noted. Preparations of "renin" from 2 sources, hog and dog kidneys,

¹ Florsheim Fellow in Cardiology.

were used. The hog kidney preparation possessed a potency such that when a dose of 0.65 mgm. dried substance per kilo of assay animal was injected intravenously a mean initial response of 38 mm. rise in blood pressure was obtained. The effects in animals anesthetized with ether, morphine sulphate-ether, sodium barbital, and in pithed animals were compared. The effect of the interval elapsing between successive injections was studied, as were also the effects of species differences in the sources and dosage differences in the amounts of "renin" used.

In the greater majority of instances the phenomenon of tolerance was observed and appeared to be unaffected by the above factors. This must therefore be considered in the interpretation of assay results where successive injections of potent pressor material are made into assay animals.

Vitamin B complex and insulin tolerance. A. R. McINTYRE and J. C. BURKE (by invitation). Department of Physiology and Pharmacology, University of Nebraska Medical College, Omaha.

The investigation of the rôle of the vitamin B complex upon the insulin tolerance of the rat has been extended to an examination of the effects of nicotinic acid, crystalline B₆, and a new lipid fraction obtained from rice polishings.

Following the injection of a standard dose of crystalline insulin (1 gamma per 10 grams of body weight) the blood sugar of the fasting rat (18 hrs.) was determined hourly for 6 hours by the Hagedorn-Jensen method. The standard diet of the control animals consisted of sucrose, flavin-free casein, lard, cod liver oil, vitamin B₁ (10 gamma per rat per day) and salt mixture. This diet is free of all known heat stable vitamin B substances and was previously shown by the authors to lower the insulin tolerance of rats (J. Pharmacol. and Exper. Therap. **64**: 465, 1938). A series of 6 animals received as a supplement to the control diet 10 gamma of crystalline B₆ (Lepkovsky) per rat per day. After three weeks on the diet the rats showed the same tolerance for insulin as the controls as judged by the blood sugar curves, i.e., the rats did not regain the tolerance for insulin shown by rats on a normal diet. Another series of 6 rats received 1 mg. of nicotinic acid per gram of food per day as a supplement to the control diet. After three weeks on the diet the rats showed a significantly greater insulin tolerance than the controls, i.e., their insulin tolerance was normal. A third series of 6 rats received 1 per cent of the lipid material from rice polishings (Supplee) as a supplement. This series appeared to have normal insulin tolerance after three weeks on the diet. It would appear from these results that the heat stable portion of the vitamin B complex which augments the tolerance for insulin in the rat as measured by the degree of hypoglycemia following insulin injections is contained, at least partially, in nicotinic acid and the new lipid fraction.

Effect of cobra venom on kidney and liver functions. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Large doses of cobra venom solution were daily administered to ten rabbits for periods varying from six to twelve weeks, and at the beginning and end of the experimental interval, the kidney and liver functions were studied. Kidney function was followed by means of the phenolsulphonphthalein test, while liver function was determined by the bromsulphalein

method. The weight and general condition of the animals were also noted. Daily administration to rabbits of the usual therapeutic dose for man (five mouse units) produced no impairment of either kidney or liver function of the animals over intervals as long as twelve weeks. Some of the rabbits received a total of five hundred mouse units. Histological examination of sections of some of these animals, which were etherized and killed after such prolonged treatment with daily large doses of cobra venom, revealed no appreciable pathological change in heart, kidney or liver, as compared with those of normal rabbits.

Comparison of menthol and its isomers. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

The pharmacodynamic action of ordinary solid menthol, U.S.P., which is laevo-rotatory, was compared with that of its isomers, d-neo menthol and d-iso menthol, and of "liquid" menthol (Swann & Co.), a preparation consisting of approximately equal parts of the three isomers. Tests were made on seedlings of *Lupinus albus* and on goldfish placed in hydro-alcoholic solutions; on mice, rats and guinea pigs injected intraperitoneally with oily solutions; on rabbits to which pure liquid menthol, as well as olive oil solutions of all three isomers, respectively, was administered by stomach tube; and on cats injected intravenously with dilute hydro-alcoholic solutions. The laevo-rotatory variety was more toxic than the d-isomers for both animals and plants. Liquid menthol, or mixture of the three isomers, differed but slightly from the official menthol in toxicity. Ten c.c. per kilo of a 5 per cent solution of liquid menthol in oil were repeatedly given to rabbits by stomach tube without permanent damage. Five c.c. per kilo of pure liquid menthol was the m.l.d. for rabbits. Solutions of 1:1,000 in alcohol, 5 per cent, intravenously injected in cats, effected respiratory paralysis, secondary depression of the circulation, and death. The lethal dosage of liquid menthol for cats was 37.4 mgm. per kilo, equivalent to the sum of its component isomers, while the lethal dose of similar solutions of menthol, U.S.P., was 34.3 mgm. per kilo weight of cat. Both menthol, U.S.P., and liquid menthol, dissolved in ethanol, effected local anesthesia. Pure liquid menthol itself is a pronounced local anesthetic. Alcoholic solutions of both menthols, liquid and U.S.P., applied to the skin of mice, rats and guinea pigs, were promptly absorbed, producing systemic effects. Pure liquid menthol also rapidly penetrated the intact skin.

Effect of Roentgen rays on germination and growth of some seeds. DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

The effect of various dosages of x-rays was studied on the germination and growth of seeds of *Lupinus albus*, tomato, wheat, oats, sunflower and squash. The physical apparatus employed consisted of a constant potential electric transformer with water-cooled therapy tubes, having a kilovoltage of 264, and 30 milliamperes. The rays were filtered through copper and aluminum plates, each one millimeter thick. The seeds were irradiated at a distance of 50 centimeters, the dosage per minute being 105r.

Germination of seeds exposed to doses of 300r and 500r was stimulated. Larger doses were less stimulating and a dosage of 1000r was actually

harmful. Germination of seeds of other plants and seedling growth were either stimulated or inhibited according to the dosage of x-rays administered. Exposure to 400r usually stimulated subsequent germination and growth of tomato, wheat, oats, sunflower and squash seeds. Larger doses inhibited and sometimes killed the seedlings. Of special interest is the relation of a rest period to growth of x-rayed seeds. When these seeds were planted a day or two after irradiation, the stimulating effect of small doses (400r) was very evident. Seeds which were not planted until several weeks after exposure to the same dose of x-rays showed no change in germination and growth. This observation may explain in part the discordant results obtained by various investigators with regard to effect of x-rays on germination of seeds.

Effect of some vitamins on growth of Lupinus albus seedlings. DAVID I. MACHT and MARY L. GRUMBEN (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Root growth under standard conditions of *Lupinus albus* seedlings in plant-physiological solution was compared with that of such seeds placed in similar solutions containing respectively vitamins A, B₁, C and D. Vitamins A and D, being very little soluble, were first dissolved in ethanol, 95 per cent, and then diluted to contain one per cent alcohol in plant-physiological saline. The water soluble vitamins B₁ and C were employed in concentrations of from 1:10,000 to 1:10,000,000. Root growth was stimulated by solutions of A and D and inhibited by 1:10,000 to 1:25,000 concentrations of B₁ and C. Very dilute solutions of B₁ and C, however, accelerated growth. Mixtures of the vitamins in plant-physiological solution led to interesting results. Certain mixtures effected synergism (i.e., a potentiation of toxicity); others exhibited antagonism; and still others, a summation of the effects of the respective vitamins. These findings agree with those of Tislowitz (Science Progress 32: 290, 1937) and others studying effects of vitamin mixtures on animals and speak against administration of promiscuous vitamin combinations to patients.

Comparison of effects of cobra venom and opiates on vision. DAVID I. MACHT and MOSES B. MACHT (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Tests for acuity of vision were first made with standard charts read at a distance of 20 feet or 6 metres. Measurements of the field of vision for white, blue, red and green were made with a self-registering perimeter before and after injection of the respective drugs. Controls were made with injections of saline solution. Such studies were made on thirty odd normal individuals. Acuity of vision was usually unaffected by injection of morphine, codeine, dilaudid or heroin although definite myosis was noted, particularly after administration of the first two drugs. Cobra venom produced no change in size of pupil but actually improved acuity of vision in nearly all the subjects. This increase in acuity of vision was indicated by a range from the normal V - 1 ($\frac{3}{8}$ ft.) to V - 1.5 ($\frac{3}{8}$ ft.) or occasionally even to V - 2 ($\frac{3}{8}$ ft.).

Effect on field of vision was even more striking. Morphine and other opiates greatly decreased the field of vision, their greatest effect being

observed with red and green colors. Cobra venom definitely *widened* the field of vision, not particularly for white because of the normally wide field for that color, but strikingly for red and green and to a lesser extent for blue.

Comparative studies of cobra venom and opium alkaloids on audition. DAVID I. MACHT and MOSES B. MACHT (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Quantitative studies on acuity of hearing for ten different tones ranging in frequency from 128 to 11584 vibrations were made with the latest model of Maico audiometer. The hearing threshold for air conduction was determined in a series of normal healthy human subjects before and after administration of various drugs, the normal threshold having been determined by repeated readings in each experiment. Cobra venom, morphine and codeine were given by injection while quinine and various antipyretics were administered by mouth; and subsequent readings were made at various intervals. Codeine, acetyl salicylic acid, acetanilid and quinine definitely but not markedly decreased acuity of perception. Much more striking were the results obtained in subjects injected with morphine sulphate and cobra venom, respectively. Twenty minutes after injection of morphine, $\frac{1}{8}$ grain, a marked dulling of hearing was noted. The decrease in acuity in susceptible individuals ranged from five to twenty decibels for various tones. The higher frequencies were the most profoundly affected. Injection of moderate therapeutic doses of cobra venom (4 to 6 mouse units), however, effected a clear-cut stimulation, or increased acuity of hearing ranging from 4 to 10 decibels for certain tones. Long-range experiments on normal thresholds of audition as well as of controls made with saline injections and with masking the sounds produced by the audiometer definitely established the clear-cut difference in action between morphine and cobra venom. These tests ran parallel to experiments on acuity and field of vision previously described.

Effect of cobra venom and opiates on mental efficiency tests. DAVID I. MACHT and MOSES B. MACHT (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

The effect of morphine ($\frac{1}{8}$ gr.), codeine ($\frac{1}{8}$ gr.), dilaudid ($\frac{1}{32}$ gr.) and heroin ($\frac{1}{4}$ gr.) was compared with that of cobra venom (five mouse units) on the mental performance of twenty-five normal individuals, college students for the most part. Three sets of arithmetical problems of progressive intricacy to be solved mentally as quickly as possible were given to the respective subjects. Each was required 1, to add seventeen to each of a series of two-digit numbers; 2, to multiply each of a second series of numbers by three and add four to the result, and 3, to add four to each of a third series of numbers, multiply the result by three and subtract thirteen. All the opiates, particularly morphine, greatly prolonged the reaction time although the number of errors was not increased in some cases. One hour after injection of five mouse units of cobra venom, there was a definite stimulation of mental activity, indicated by much shorter reaction time and fewer errors, as proven by statistical analysis and determination of critical ratios. The narcotics definitely depressed all the

subjects, while cobra venom either produced no subjective symptom whatever or brought about a brightening of the intellect not unlike that effected by caffeine.

Influence of cobra venom and morphine on muscle work. DAVID I. MACHT and MOSES B. MACHT (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Experiments were made on twenty normal individuals (college students) with Porter's ergograph (W. T. Porter: Introduction to physiology, 1901, p. 220). The tonicity and strength of voluntary contractions of the abductor muscle of the index finger for five minutes was measured before and after administration of the drugs and registered on a kymograph. Morphine ($\frac{1}{4}$ gr.), codeine ($\frac{1}{4}$ gr.) and dilaudid ($\frac{1}{32}$ gr.) had little or no effect on subsequent performance of the abductor muscle. Occasionally a weakening of power was observed in susceptible individuals. Injection of five mouse units of cobra venom usually increased both the height of contraction and tonicity of muscle quite definitely as indicated by kymographic tracings.

Effect of cobra venom and other analgesics on the tapping test. DAVID I. MACHT and MOSES B. MACHT (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

The effect of morphine, codeine, heroin and dilaudid, amidopyrine, the salicylates, barbiturates and other analgesics, as well as of cobra venom, on neuromuscular coördination and work performance of muscles of the arm and hand was tested by the Dunlap tapping apparatus. By this method the number of taps made per unit of time with a stylus on a metal plate was registered electrically. The normal performance was first determined by taking the average of the figures obtained in three successive minute intervals of tapping and was then compared with the results obtained after administration of various drugs. All the narcotics and also the antipyretics definitely retarded muscular performance of most of the subjects, as shown by the decreased number of taps. Caffeine taken in the form of tea, etc., increased efficiency in tapping. Cobra venom injections also definitely increased the rate of tapping in the majority of subjects.

The basic patterns of periodic potentials found along the respiratory arcs.

CONWAY S. MAGEE (by invitation), JOHN W. BRICKER (by invitation) and ROBERT GESELL. Department of Physiology, University of Michigan, Ann Arbor.

Periodic respiratory potentials in phase with respiration were recorded at important peripheral and central stations of the respiratory arcs.

Three characteristic patterns of fusillades were found: 1, the "slowly augmenting" type in which the deflection rises slowly during the inspiratory phase of breathing and falls abruptly with the onset of expiration; 2, the "rapidly augmenting" expiratory pattern which rises suddenly and then declines slowly as expiration proceeds, and 3, the "steady state" pattern, roughly rectangular in shape, which continues with relatively uniform intensity throughout the expiratory phase.

Frequency of firing of unicellular discharges when plotted against time yielded curves of the characteristic shapes found in the fusillades.

These patterns of multicellular and unicellular discharges were found throughout the course of the respiratory arcs in muscle, motor nerves, anterior horns, reticulospinal tracts, reticular nuclei, reticular formations, internal arcuate fibers, parafascicular grey, the cuneate and gracile nuclei, the dorsal columns of the cord, and the peripheral vagal trunks.

Statistical analysis establishes the slowly augmenting type as inspiratory and the rapidly augmenting and steady state types as expiratory. Over 96 per cent of the inspiratory potentials fall into the slowly augmenting type. Over 97 per cent of the expiratory potentials fall into the rapidly augmenting and steady state patterns, 54 per cent and 43 per cent respectively.

Interrelations of inspiratory and expiratory centers in the cat. H. W. MAGOUN, R. F. PITTS and S. W. RANSON. Institute of Neurology, Northwestern University Medical School, Chicago, Ill.

In an accompanying abstract, Pitts, Magoun and Ranson have presented evidence which indicates that the tonic inspiratory and expiratory responses, induced by electrical stimulation of a localized region of the reticular formation of the medulla of the cat, represent the activation of inspiratory and expiratory divisions of what has been called the respiratory center.

Additional study has shown that combined weak stimulation of the inspiratory centers in either half of the medulla yielded responses greater than the sum of the reactions obtained from either half alone, and with stronger currents maximal inspiration was obtained from one half of the medulla, no added effect resulting from combined stimulation of the other half. Direct spread of current was negligible, and some arrangement facilitating the spread of excitation within the center must be present.

Combined stimulation of inspiratory and expiratory centers revealed that each exerted a reciprocal inhibition upon the effects of the other, the inspiratory influence being dominant. With various combinations of stimuli this reciprocal inhibition could be utilized in driving respiration in a number of ways.

Inspiratory cramp or apneusis, induced by pontile decerebration and blocking the vagi, closely resembles the responses to stimulation of the inspiratory center. Stimulation of the inspiratory center during apneusis increased inspiration and following stimulation the increased amplitude was maintained. After more marked stimulation the inspiratory cramp was abolished, however, and then gradually restored. It is suggested that apneusis represents the activity of the inspiratory center under conditions of release. Apneusis could be broken down into rhythmic respiration by successive stimulation of the expiratory center.

Some respiratory and circulatory disturbances of the erect posture. ROLLAND J. MAIN. Department of Physiology and Pharmacology, Medical College of Virginia, Richmond.

Hitchcock and Ferguson (Am. Journ. Physiol. **124**: 457, 1938) (1) have suggested that the low alveolar CO_2 accompanying standing is due to interference with CO_2 transport caused by stagnation of blood in the legs, with consequent diminution of pulmonary ventilation and total CO_2

output during the *early* part of standing. Therefore they claim that the lowered alveolar CO_2 cannot be explained as due to overventilation. However, since arterial CO_2 tension is regulated by the respiratory center, variations in the total CO_2 output should have no effect, so long as the respiratory system is competent. The low arterial CO_2 tension indicates that overventilation must be occurring in spite of the diminished pulmonary ventilation, apparently because the diminished circulatory blood volume requires less pulmonary ventilation to overventilate it, than did the normal blood volume to be normally ventilated.

The reason for this overventilation has been suggested as due to diminished pressure in the carotid sinus or to cerebral ischemia. I find that increasing the blood pressure by administration of epinephrine to standing subjects has no appreciable effect on alveolar CO_2 tension. Consequently it appears that the fall in pressure in the carotid sinus may not be the cause of this overventilation. This suggests that cerebral ischemia may be responsible.

The suggestion of Hitchcock and Ferguson that there may be an increased circulation rate in the upper body upon standing in order to maintain the same cardiac output as when lying, is not borne out by determinations of the circulatory rate from arm vein to throat. The values I have obtained in subjects standing and lying are practically identical. However, perhaps the total venous return from the arms is greater standing.

Studies on the genesis of ischemic pain—the influence of potassium and lactate ions. GEORGE L. MAISON (introduced by A. B. Hertzman). Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

It has recently been reported that the ischemic pain modality can be evoked by the intramuscular injection of hypertonic sodium chloride (J. H. Kellgren, Clin. Science, **3**: 175, 1938). When this modality is evoked by ischemic work the factor which stimulates the nerve endings is commonly supposed to be a chemical metabolite. It therefore was decided to determine the liminal concentration required to produce pain for each of the known products of muscular contraction. The liminal concentration of the ischemic pain factor will probably show different characteristics than those of the non-culpable substances as follows: first, the liminal concentration should be less than the maximum which can be developed by physiological activity. Second, the effective concentration should be markedly reduced by previous ischemic work insufficient to produce pain. Third, the liminal concentration should be less than isotonic.

The substances were applied through intramuscular needles in a volume of 0.3 cc. Isotonicity of the fluid was maintained by the addition of sodium chloride. The extensor digitorum communis was used. Ischemia was created by 220 mm. Hg pressure and work done on an ergograph with a load of 600 grams.

Potassium in the form of KCl produced the following results: In 11 subjects the limen varied from 120 to 360 mgm. per cent K ion. In the normal muscle the average limen was 225 mgm. per cent. In the ischemic muscle without work 210 mgm. per cent. In the ischemic arm after work ($\frac{1}{2}$ of that required to produce pain) the average limen was 220 mgm. per cent. The limen thus remained the same.

Sodium sarcosylate solution containing approximately 1800 mgm. per cent of lactate at pH 7 was not effective in producing pain in any of 10 subjects whether the arm was worked or not.

Of 32 subjects used 7 were unsatisfactory because 0.9 per cent NaCl was provocative of pain. Fifty per cent of the subjects felt pain in the muscle belly alone; the rest had pain referred to the hand as well.

This evidence renders it unlikely that either the K ion or the lactate ion acting intercellularly can be the ischemic pain factor.

The distribution of injected radioactive sodium and phosphorus in tissues.

JEANNE F. MANERY and WILLIAM F. BALE (by invitation). Departments of Physiology and Radiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

Radioactive sodium (Na^{24}) and phosphorus (P^{32}) were used to investigate the distribution and the rate of entrance of sodium and phosphate into the intra- and extracellular phases of mammalian tissues. Both Na^{24} and P^{32} (the latter as Na_2HPO_4) were injected in isotonic sodium chloride solutions. Eight rats and 3 rabbits were sacrificed at various periods after intraperitoneal or intravenous injections and the tissues were analysed for sodium, chlorine and radioactivity. Radioactivity was determined on a Geiger-Müller counter with an average per cent difference between duplicates of 7 per cent.

In 20 minutes after injection the radioactive sodium absorbed became distributed in the same concentration as in plasma throughout 25-30 per cent of the body weight with no further change in 2 hours. This value agrees with the estimates of the volume of the extracellular phase of mammals obtained by other methods. Radioactive phosphorus however, spreads throughout the entire body in 20 minutes, after which it gradually leaves the plasma to become concentrated in tissues.

Of particular interest is the fact that injected radioactive sodium spreads quickly from the plasma into individual tissues in the same proportion as total sodium is distributed. Analyses showed that the volume of the following tissues penetrated by radioactive sodium in 20 min. to 1 hour was identical to the sodium space determined chemically; skin, tendon, adipose tissue, kidney, testes, cartilage, spleen, liver, stomach mucosa and muscle, intestine, heart, skeletal muscle, placenta and uterine wall. In many of these, e.g., cartilage, testes and connective tissues the sodium space differs considerably from the chloride space (Manery and Hastings, XVI Internat. Physiol. Congress II, 274). Brain and sciatic nerve were outstanding exceptions because even in 1 hour the radioactive isotope had penetrated only a small fraction of the total sodium space.

Hence, sodium which is characteristic of body fluids and connective tissues is distributed and confined to a volume which must closely approximate the extracellular phase. Phosphate on the other hand, being a cellular constituent quickly enters the intracellular phase and is concentrated in the cells of individual tissues where it is actively metabolized.

Microscopic observations upon the pacemaker of the isolated terrapin sinus venosus. FRANK MARESH (introduced by W. J. Meek). Physiology Department, University of Wisconsin Medical School, Madison.

The sinus venosus and large veins were removed from the body of a terrapin, detached from the auricles and ventricle, stretched upon a metal

frame, mounted on a glass slide, and observed under the microscope. The pacemaker consists of automatic fibers which are elongated, cross-striated, fusiform, and well differentiated from the surrounding tissues. The automatic fibers are gathered into bundles by a thin connective tissue membrane and send individual fibers to other bundles or fibers, forming an intricate, endless system of dividing bundles and interlacing fibers which covers not only the sinus venosus but extends over the large veins. The automatic fibers are actively contractile and, in turtle serum, will continue their activity for as long as six days. The complicated network lies in the tunica media and can be found 2 cm. from the auricle.

The automaticity is not limited to the fibers at the sino-auriculo junction, for narrow rings, strips or sections isolated from any part of the sinus or large veins reveal contractions which continue uninterruptedly in serum, in Ringer's solution, in stains and in a large variety of ionic concentrations. Not any of the usual laboratory stains penetrated the automatic fibers; however, in green stains (naphthol green, fast green) the preparations were the clearest, showing such details as the myofibrils, Hensen's line, z disks, mitochondria.

Weak alkalies (NH_3) and oxidizing agents (KMnO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$) stopped the contractions quickly, but in a saturated boric acid solution the contractions continued for hours. Adrenalin increased the length of excursion of the contraction but not the rate of the contraction.

The impulses for automaticity did not jump transversely from one bunch of fibers to another bundle but did spread along the longitudinal axis of the fibers. Any interference with the spread of the impulse along the longitudinal axis produced any variety of blocks or independent rhythms between different regions. Adrenalin abolished such irregularities.

An isolated active pacemaker from a terrapin sinus venosus under the lens of a microscope. FRANK MARESH (introduced by W. J. Meek). Physiology Laboratory, University of Wisconsin Medical School, Madison. (Demonstration.)

A segment of an active portion of the sinus venosus will be stretched on a metallic frame, immersed in a naphthol-green solution, mounted under a coverslip, and observed under a microscope.

Inhibition at a sympathetic synapse. AMEDEO S. MARRAZZI (introduced by George B. Wallace). Department of Pharmacology, New York University College of Medicine, New York City.

Abnormally large amounts of acetylcholine, either injected or caused to accumulate by applying strong stimuli to the preganglionic trunk of the eserized preparation, are known to depress the ganglionic response. This inhibition is of doubtful physiological importance because of the excessive doses necessary. The following experiments suggest the presence of a more significant, synaptic inhibitory mechanism, which is adrenergic in nature.

In cats and rabbits anaesthetized with nembutal, postganglionic potentials were recorded as a sensitive and accurate index of impulses which had traversed the ganglionic synapses. Adrenaline was injected intravenously in amounts small enough to be of physiological significance (5 gamma in 4 kgm. animals). This quickly reduced the ganglionic

response elicited by constant, submaximal shocks applied at 2/sec. to the preganglionic trunk. In any dose used (5-500 gamma) this inhibition was never preceeded by facilitation.

The action of adrenaline was not dependent upon any vascular change it might have produced. This was shown by the effects of complete interruption of the circulation to the ganglion. No change took place in the first 5 minutes. Facilitation occurred usually in the next 5 minutes, while a decline to extinction began only after 10 minutes.

It did not seem feasible to subdivide and explore the preganglionic trunk for inhibitory fibers, and the contribution of such inhibitory fibers could not be distinguished in the ganglionic excitation produced by electrical stimulation of the whole trunk. However, I have found that another sympathomimetic drug, ephedrine, has a similar inhibiting action on the ganglion. If it may be accepted that ephedrine acts in this case either by stimulating inhibitory sympathetic nerve ends, thus liberating an adrenaline-like neurohumor, or else by protecting from destruction this neurohumor formed during their stimulation, then inhibitory nerves must be present.

Adrenaline, which either acts as or is closely related to a neurohumor is found to produce inhibition at a sympathetic synapse, in physiologically small as well as in larger quantities. Indications of the functioning of inhibitory fibers exist. The findings suggest the existence of an adrenergic, neurohumoral, inhibitory mechanism in a sympathetic ganglion.

Relation between electrolyte imbalance and excretion of an antidiuretic substance in adrenalectomized cats. S. J. MARTIN, H. C. HERRLICH (by invitation) and J. F. FAZEKAS (by invitation). Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, N. Y.

Recently Gilman and Goodman and Ivy et al. reported the excretion of an anti-diuretic principle in the urine of dehydrated rats. Since dehydration occurs in adrenal insufficiency, it appeared significant to determine 1, the excretion of such a substance during hypoadrenia, and 2, its possible relationship to electrolyte imbalance.

The daily fluid intake and output was recorded in 11 cats, and during and after cessation of a maintenance dose of cortin following adrenalectomy. Daily bovev rations were constant and, except in dehydration experiments, water was given ad lib. The presence of an anti-diuretic principle in urine was assayed by a modified technique of Burn in 168 male rats. Blood was drawn for serum sodium and potassium analyses with each urine collection. The results are summarized as follows:

1. We confirmed previous findings that an anti-diuretic principle is excreted in the urine of dehydrated rats and cats but not in that of non-dehydrated controls.

2. The excretion of this principle in 8 dehydrated cats (24-72 hours) was not accompanied by significant variations in serum sodium or potassium.

3. An anti-diuretic substance was excreted in the urine of adrenalectomized cats 24 hours after cessation of maintenance doses of cortin. This was chiefly due to decreased water intake. The appearance of the anti-diuretic principle, with one exception, preceded any significant change in serum sodium and potassium. It was found in much larger quantities

in 48 and 72 hour samples when a low serum sodium and occasional high potassium were noted. This principle was not excreted in the urine of adrenalectomized cats maintained by cortin.

4. Decreased serum sodium induced by intraperitoneal glucose injection and abdominal paracentesis in 4 cats resulted in excretion of larger quantities of anti-diuretic substance than found in adrenalectomized cats 72 hours after the last injection of cortin.

5. It is believed that in hypoadrenia there is an initial stimulation of the posterior lobe of the pituitary associated with early dehydration. This action is augmented secondarily by electrolyte imbalance of the blood.

Endocrine influences on the excretion of prostatic fluid. M. H. MASINA (by invitation), C. HUGGINS and LILLIAN EICHELBERGER. Department of Surgery, University of Chicago, Ill.

A simple method was developed for the quantitative collection of prostatic excretion in dogs, allowing determinations to be made every two or three days for six months. The stimulating agent was pilocarpine hydrochloride 3-6 mgm. given intravenously; the dogs remain in a healthy condition.

The prostatic fluid decreased in amount or ceased entirely in all dogs within several weeks after entering the laboratory. This could not be prevented by vitamin supplements to the diet. Prostatic excretion was restored or augmented by testosterone propionate injections and the amount of fluid obtained was related to the amount of testosterone injected.

In a series of dogs, following bilateral orchietomy, testosterone injections were given until an equilibrium was reached and various endocrine modifications were then carried out. Thyroparathyroidectomy was without effect. Bilateral adrenalectomy caused a decrease in the amount of fluid produced. Hyperthyroidism produced wide fluctuations in the amount of fluid obtained.

The electrolyte concentration and pattern of the fluid were nearly constant under all of the conditions of the experiments. The fluid was isotonic and the following ionic concentrations were found: sodium 160 milli-equivalents, $\sigma \pm 2.8$; potassium 5.22, $\sigma \pm 0.24$ milli-equivalents; chloride 162, $\sigma \pm 3$ milli-equivalents. The fluid is highly unbuffered.

The blood clotting time of adrenalectomized rats during adaptation to various agents. G. MASSON (introduced by Hans Selye). Department of Anatomy, Histology and Embryology, McGill University, Montreal, Canada.

While some investigators observed an increase in the blood clotting time, others claimed to have obtained opposite results after adrenalectomy. Our previous experiments have shown that under the influence of non-specific damaging agents, the coagulability of the blood increases and that the fibrin formation is particularly rapid when the symptoms of the alarm reaction are optimally developed. We felt therefore, that possibly the changes in the clotting time of adrenalectomized animals might also be dependent on the degree of damage inflicted upon them. In order to settle this question, twenty-four adult male hooded rats, weighing 180-242 grams were adrenalectomized. Twenty-four hours later, six of them were exposed to cold (+3 to +5°C. during six hours daily). Six were treated with three daily injections of 0.2 cc. of a 4 per cent formaldehyde solution

and a third group of six was forced to exercise for 15 minutes, three times daily in drum cages having a diameter of 12 inches and revolving at the rate of 18 revolutions per minute. The remaining six animals were left as untreated controls.

It was found that irrespective of the type of treatment used during the first twenty-four hours of the experiment, all treated animals showed a decrease in the clotting time as evidenced by a more rapid formation of fibrin. At this time the fibrin formation occurred within a few seconds as compared with 1-3 minutes in normals or untreated adrenalectomized animals. After two to three days of treatment when the animals entered into the resistant stage, the fibrin formation time increased and reached almost the original level. A few days later however, when the animals became exhausted, a second decrease in the time of fibrin formation occurred and soon after this, most of the rats succumbed. In distinct contrast to the treated animals, the fibrin formation of the untreated adrenalectomized controls remained unchanged in most cases and in a few instances pre-mortally it actually rose above the normal level. From our experiments, it appears that during the stage of the alarm reaction and the stage of exhaustion of the "general adaptation syndrome" (as described by Hans Selye, *Proc. Soc. exper. Biol. and Med.* **38**: 728, 1938), the fibrin formation becomes very rapid in the adrenalectomized animal but no such change is seen in adrenalectomized rats otherwise untreated or in those which following an alarm reaction entered into the "stage of resistance."

The effect of the anterior pituitary extract upon the deposition and character of the body fats in the fowl. W. A. MAW (by invitation), J. A. STUART (by invitation) and J. B. COLLIP. Department of Poultry Husbandry, (Macdonald College), and the Department of Biochemistry, McGill University, Montreal, Canada.

Mature cockerels have been injected intraperitoneally, over a twenty-one day feeding period, with anterior pituitary extract no. 622c, and the effect upon the amount and character of the body fat determined. The extract increased the amount of abdominal fat, but greatly decreased the carotinoid pigment content of the fat. While no difference in feed consumption was noted between the injected and control groups, a difference in gain in body weight approaching significance was shown in favour of the injected birds.

Relationship of intramuscular tissue pressure to syncope induced in man by gravity. H. S. MAYERSON and G. E. BURCH (by invitation). Departments of Physiology and Medicine, Tulane University School of Medicine, New Orleans, La.

Repeated observations on 7 trained young adult males suggest that low intramuscular tissue pressure is a significant underlying factor in the development of syncope induced by the upright position.

The experiments were conducted in a constant temperature room (75°F; humidity = 50 per cent) on three individuals who consistently manifested syncope when in the upright position, three who showed no embarrassment and one borderline subject who showed signs of syncope on some occasions but not on others. Measurements were made on intramuscular tissue pressure in the right gastrocnemius at intervals of 15 to 60 seconds.

After constant levels had been obtained in the horizontal position the subjects were tilted to an angle of 75° , being suspended on the table by supports over the iliac crests. The absence of a foot-rest afforded maximum muscular relaxation with little or no impairment to the circulation of the lower extremities.

The intramuscular tissue pressure response when syncope develops in the upright position is strikingly different from that when there is no embarrassment. The "fainters" show a lower mean level (7.6 cm. H_2O) in the horizontal than the "non-fainters" (10.7 cm.) but in both types there is usually an immediate rise of 3 to 6 cm. on tilting. When the upright position is well tolerated, the pressure continues to rise (mean maximum value = 24.8 cm.). In contrast, when syncope is manifest, this secondary rise is absent or, in some cases, the pressure actually drops below horizontal control levels before collapse. The close association between muscle tissue pressure and syncope is well illustrated by the borderline subject who, when the pressure reached 16.4 cm., showed only slight dizziness when suspended for 37 minutes, but when the maximum pressure was only 11.5 cm., manifested syncope at the end of 20 minutes. Furthermore, when two "fainters" stood on their toes on a foot-rest and the tissue pressure as measured in the gastrocnemius reached 53.5 and 73.7 cm. respectively, they showed no signs of syncope during periods which exceeded considerably those when the levels were low and manifestations of syncope developed.

Certain characteristics of injury potential in cardiac muscle. WALTER J. MEEK, J. A. E. EYSTER and HAROLD GOLDBERG (by invitation). Physiological Laboratory, University of Wisconsin, Madison. (Read by title.)

This work represents an effort to determine some of the factors relating to the production of the large positive potentials which develop in an injured region of heart muscle when the surrounding normal muscle enters into activity (iap). Local areas of injury were produced on the surface of the exposed hearts of dogs and turtles by the suction electrode of H. C. Wiggers.

That the production of the iap depends on activity of the surrounding muscle is shown by the fact that it cannot be produced in the auricular appendage separated functionally from the auricle. In this preparation the negative resting potential of injury (irp) of the usual magnitude is readily produced, but no positive phase develops. On the other hand the iap does not appear to depend on contraction of the muscle surrounding the injury, since it occurs in almost the usual magnitude and form in a region of the ventricle rendered non-contractile by local ischemia. An ischemic region without further injury develops a modified iap. In bipolar leads from two regions on the ventricular surface, one injured, the other uninjured, the production of ischemia in either region modifies the iap curve, but the effect is greater when the previously uninjured region is rendered ischemic. In the latter case, the situation is comparable to that of two injured regions, and results in potential time curves of various form and magnitude.

The relative time of onset of the iap in different regions of the surface of the dog's ventricle accords with the onset of activity as indicated by other methods of study. The earlier the onset the slower is the maximum

positive potential reached and the latter thus tends to occur at nearly the same time at the various points on the ventricular surface.

*Intralumen pressures of the pyloric antrum and duodenal bulb.*¹ I. MESCHAN (by invitation), D. A. BRODY (by invitation) and J. P. QUIGLEY. Western Reserve University Medical School, Cleveland, O.

We have employed optical registration from a small open tube or from a small balloon encased in a perforated metal capsule to obtain continuous records which accurately measure gastro-intestinal intralumen pressures. Changes in intralumen pressure in a portion of the gut are the resultant of modifications in the gut wall tension at or near that region and the resistance to escape of contents from that region. Records obtained by the balloon-water manometer technic are modified to an unpredictable extent by: 1, directly transmitted changes in gut wall tension; 2, volume changes in the gut; 3, stretching of the gut by the balloon; 4, inadequate vibration frequency of the recording system. These objections are essentially absent in our optical registration.

Our pressure recorders were introduced into the pyloric antrum and duodenal bulb of normal unanesthetized dogs provided with permanent metal cannulae (technic described by Meschan and Quigley, *Am. J. Physiol.* 121: 350, 1938). In fasting animals, the basic pressure of the antrum and bulb closely approximated zero. Maximal pressures varied greatly; rising as high as 90 cm. H₂O but averaging about 25. Antral pressures usually exceeded bulbar pressures by 10-15 cm. H₂O. Elevation of antral pressure, preceded, occurred simultaneously with, or was unrelated to elevation of bulbar pressure. Negative pressures of 5-10 cm. H₂O were frequently obtained, especially directly following a period of elevated pressure. The pressure records were not modified by sealing the cannulae.

Basic pressures of the stomach and bulb were elevated 2-5 cm. H₂O when 200 gm. of corn meal mush was eaten but were unaltered when 50 cc. of distilled water or 0.9 per cent saline entered the stomach through the cannula. Following such introduction of material into the stomach, irregular pressure changes occurred for 2-3 minutes. Subsequently, the pressure changes were over a smaller range, especially in the case of mush, and were less frequent than preceding the administration. Pressure changes of this pattern continued during the period material was being discharged from the stomach. Interpretations of these results will be presented.

The physiology of the corpus striatum. FRED A. METTLER, HARLOW ADES (by invitation), ELI LIPMAN (by invitation) and E. A. CULLER. Department of Anatomy, University of Georgia School of Medicine, Department of Psychology, University of Illinois, and Department of Psychology, University of Rochester, Rochester, N. Y. (Read by title.)

Direct stimulation of various portions of the corpus striatum either by means of electrodes carried in stereotaxic instruments or directly, following ablation of overlying cortex, has failed to yield significant indications of function. Nearly all previous experimenters have however carried

¹ Aided by a grant from the Council on Pharmacy and Chemistry, American Medical Association.

out such stimulation in the absence of motor activity. Recognizing the frequent association between an exacerbation of extrapyramidal dysfunction and increased volitional activity the present workers undertook to investigate the effect of stimulation of the corpus striatum upon movements already in progress.

The animals were prepared by exposing the cortex and fastening the head in a stereotaxic instrument carrying a concentric needle electrode. Stimulation of various portions of the corpus striatum by means of the needle electrode never gave any result when the corpus striatum was excited alone but if a movement were first induced by stimulation of the motor cortex (by a wandering electrode) and then the corpus striatum was excited several new findings were encountered. The basic movement which was studied was phasic forepaw flexion. Stimulation of the caudate nucleus while such a movement was in progress always resulted in its complete inhibition beginning with a decrease in amplitude and frequency of the phasic pattern. Such inhibition persisted for some time after caudate stimulation had ceased. Stimulation of the putamen gave essentially the same result as that of the caudate. The globus pallidus effect was different however. Stimulation of this structure in the course of a cortically induced movement resulted in an holding of the forepaw in a state resembling, if not identical with, plastic tonus.

Other extrapyramidal mechanisms were investigated. It was observed that stimulation of the substantia nigra alone produced an increase in extensor tonus and that in conjunction with cortical stimulation a marked tremor resulted. Corpus subthalamicum (alone) stimulation results in contraction of the dorsal midline musculature of the opposite side. The tract producing this effect decussates just oral to the red nucleus. A positive motor effect which could be definitely allocated to the red nucleus was never obtained in any of our cases.

Further studies upon the blood-cerebrospinal fluid barrier of normal white and negro children. FRED A. METTLER, W. R. BROWN (by invitation), MEINHARD ROBINOW (by invitation) and CLAUDE McK. BURPEE (by invitation). Child Neurology Research (Friedsam Foundation), Departments of Anatomy, Biochemistry and Pediatrics, University of Georgia School of Medicine, Augusta. (Read by title.)

Last year a paper was presented to the members of the American Physiological Society at Baltimore in which it was pointed out that the blood-cerebrospinal fluid barrier as measured by the bromide permeability quotient and determined by the Katzenelbogen modification of the Walter method differed substantially in white and negro children.

During the past year this phenomenon has been checked by the use of electrical titration instead of the Walter method. The results obtained have been in agreement with our findings as reported in 1938, i.e., the permeability quotients of normal negro children fall into lower brackets than those of normal white children. No explanation has as yet been encountered for this finding but preexisting variation in blood chlorides may be ruled out as an operative factor.

The effect of oral and subcutaneous administration of thyroxine on the metabolism and heart rate of thyroidectomized rats and the physiologic action of a thyroid hydrolyzate after extraction of the thyroxine moiety. A. E.

MEYER and M. THOMPSON (introduced by A. A. Hellbaum). Research Laboratory of the Maltine Company, Brooklyn, N. Y.

The thyroidectomized rat is about 25 times more sensitive in its metabolic response to thyroxine than the normal rat, and because of the higher uniformity in the reactions, serves as a preferable test animal for the metabolism stimulating effect of thyroid preparations. Thyroxine was used as a standard in dosages of 0.75 γ per 10 grams given orally on three consecutive days. This produced an increase in metabolic rate of about 30 per cent, measured on the fifth day; a comparable effect also occurred when an equal dose of thyroxine was administered subcutaneously. A comparatively slight but similar increase in heart beat occurred in both types of administration.

A butyl alcoholic extract prepared from a hydrolyzate of thyroid globulin containing 0.75 per cent of iodine, produced the same metabolic increase as thyroxine when given in equivalent doses as calculated on iodine content. This latter product, however, did not stimulate the heart beat at a dosage that increased the metabolic rate by 34 to 40 per cent. Since the commercial thyroxine used contained about 1 per cent of impurities it may be supposed that the moderate stimulation of heart beat was caused by a substance still contained in this preparation but not present in the butyl alcoholic extract. Our tests permit the assumption that the latter compound contains iodine exclusively in the form of the thyroxine complex, the other constituents being iodine free substances. The aqueous residue from the extraction with butyl alcohol was precipitated with ethyl alcohol and the liquid was evaporated to dryness. The residue contained 0.35 per cent iodine. At a rather large dose (2,200 mgm. per 10 grams daily) it caused a definite increase in heart rate but no change in metabolism. The stimulation of heart rate and metabolism in thyroidectomized rats are not parallel when various substances are tried, but seem to be, to a certain extent, independent effects.

The metabolism of proteins and amino acids in inanition. A. T. MILHORAT.

Russell Sage Institute of Pathology in affiliation with New York Hospital, and Departments of Medicine and Pharmacology, Cornell University Medical College, New York City.

The effect of ingestion of proteins, amino acids and acid hydrolysates of proteins on the excretion of nitrogen, sulphur, and other urinary constituents was studied in dogs fasted for from ten to thirty days. Blood fibrin, zein, edestin, casein and peptone of casein, given in amounts of 5 grams had no effect on the composition of the urine. On the other hand, when gelatin was given, about forty per cent of the nitrogen was excreted. The addition of all the non-dispensable amino acids or of valine and methionine was followed by a large increase in the retention of the nitrogen from gelatin. However, all the other combinations of amino acids studied had no effect. When gelatin and blood fibrin were given together the extra nitrogen excreted equalled that recovered when the gelatin alone was ingested. The nitrogen of acid hydrolysates of casein and blood fibrin was largely excreted when the hydrolysates were given in one dose. When the hydrolysates were administered in small amounts over periods of several hours an increase in urinary nitrogen occurred on the first day and a corresponding decrease on the following day. The nitrogen of glycine and aspartic acid was completely eliminated, whereas

about 50 per cent of the nitrogen of certain non-dispensable amino acids, such as l-leucine, was retained. A mixture of amino acids resembling the composition of zein and lacking lysine was given in small doses. Practically all of the nitrogen was retained.

The results suggest that for the retention of nitrogen under these conditions lysine and possibly tryptophane are not needed. Moreover the rates of absorption and of delivery of amino acids to the tissues are of importance in determining the retention of the nitrogen.

The effect of prolonged exposure to high concentrations of carbon dioxide on the acid-base balance and blood cells of the dog. A. T. MILLER, JR. (introduced by Robert Gesell). Department of Physiology, University of Michigan, Ann Arbor.

Dogs were kept for periods of 1 to 6 weeks in a large metal chamber containing 1.5 to 5.0 per cent carbon dioxide. The oxygen concentration was maintained at 21 per cent in one series of experiments, and 30 to 35 per cent in another series. The following results were obtained: 1. The pH_a was lowered 0.10 to 0.15 of a unit. 2. The arterial CO_2 combining power increased during the first week and then decreased to a value below normal. 3. There was a transfer of water and chloride from plasma to cells and of bicarbonate from cells to plasma, in accordance with the Donnan theory. 4. Whole blood chloride decreased. This change is interpreted as a transfer of chlorides from plasma to tissue fluid. 5. The erythrocyte count was increased 15 to 20 per cent. 6. There was no evidence of hemo-concentration (plasma protein concentration remained constant), and the erythrocyte increase was obtained in splenectomized animals. Accordingly, it is interpreted as a true bone marrow response. 7. The increase in hemoglobin was somewhat less, averaging 10 to 15 per cent, and lagged behind the erythrocyte increase, due to release from the bone marrow of immature cells deficient in hemoglobin. 8. The reticulocyte count increased from a normal value of 0.2 to 0.4 per cent to a value of about 10 to 15 per cent. 9. There was an exceedingly variable increase in the leucocyte count, ranging from 10 per cent or less to more than 200 per cent, and averaging 85 per cent. There was a slight increase in the neutrophil: lymphocyte ratio.

The rôle of the liver in ethyl alcohol oxidation. I. ARTHUR MIRSKY and NORTON NELSON (by invitation). The May Institute for Medical Research of the Jewish Hospital, Cincinnati, O.

In an attempt to determine the validity of the hypothesis that the oxidation of ethyl alcohol is dependent upon the simultaneous oxidation of carbohydrate, a series of studies were performed with dogs, rabbits and man.

Our data reveal that ethyl alcohol is not utilized by the muscles in the absence of the liver; that neither the amount of available carbohydrate nor the administration of large amounts of insulin has any influence on the oxidation of alcohol in the absence of the liver. Furthermore, the utilization of alcohol is not significantly impaired in the depancreatized dog receiving no insulin, thus indicating that insulin is not essential for the oxidation of alcohol even in the presence of the liver.

In an effort to determine some of the factors influencing the oxidation of alcohol by the liver, a study was performed in which the rate of alcohol

removed from the blood was determined in rabbits immediately after the ablation of various amounts of liver. A striking relationship was observed between the per cent of liver removed and the amount of alcohol utilized, the coefficient of correlation between them being above -0.9 . This suggests that the oxidation of alcohol is dependent upon the amount of normal liver present in the organism. In accord with this view is the observation that the inhalation of chloroform by dogs for various short periods of time results in a definite decrease in the rate of alcohol utilization. Preliminary observations in man also indicate a relationship between liver function and ethyl alcohol oxidation.

The influence of right intraventricular pressure conditions on the distribution and oxygen yield of blood in the coronary circulation of the isolated heart.

GORDON K. MOE (by invitation) and MAURICE B. VISSCHER. Department of Physiology, University of Minnesota, Minneapolis.

That right intraventricular pressures have an influence on the distribution of coronary blood has been previously shown by Anrep and his collaborators and by Johnson and Wiggers (1937). The latter workers measured only the coronary sinus outflow, which they found varied directly with maximum right ventricular pressure, despite concurrent changes in aortic pressure.

Studies on the isolated dog heart in which *both* the principal fractions of coronary venous blood flow were measured and their oxygen contents determined have yielded results of significance in all calculations of cardiac metabolism. We have found that the *pressure difference* between mean aortic and pulmonary pressures is the factor of importance in the distribution of flows. So long as this difference exceeds 40 mm. Hg the fraction of coronary blood draining via the right Thebesian channels remains remarkably constant, confirming Anrep's earlier work. The percentage varies between 35 and 48 for different animals, but in a much narrower range for individual hearts. A pressure difference less than 40 mm., however, is accompanied by a sharp decrease in Thebesian flow, which is partly or completely compensated by an increase in coronary sinus outflow, indicating a critical level below which right heart venous channels fail to function. Impairment of Thebesian outflow plays an obvious rôle in right heart failure accompanying increased pulmonary arterial pressure.

As would be expected, the diminution in Thebesian flow is accompanied by an increased coefficient of oxygen utilization. That is, the ratio of the coefficient for Thebesian blood to that of coronary sinus is found to bear an inverse relation to the pressure difference. In no case, even under normal pressure conditions, were the oxygen contents of both fractions of coronary venous blood found to be identical; this fact therefore invalidates determinations of cardiac oxygen consumption by the arterio-venous difference method when only the coronary sinus blood is sampled.

On the use of dried (lyophile) ascitic fluid in hemorrhagic shock. ARTHUR G. MULDER, HARRY A. DAVIS (by invitation) and ALBERT STREETER (by invitation). Departments of Physiology and Pathology, College of Medicine, University of Tennessee, Memphis.

In this study an effort has been made to determine the effectiveness of the lyophile form of ascitic fluid after hemorrhage. Previous work (Davis) has shown that unconcentrated ascitic fluid may be of value as replacement

for fluid following hemorrhage. The dried ascitic fluid redissolved readily and was used in various concentrations usually about $2\frac{1}{2}$ times the original. Cross agglutination tests were performed between the ascitic fluid and the blood of the recipient animal.

The dogs were brought to shock level by bleeding $3\frac{1}{2}$ per cent to 5 per cent of their body weight. As a result of this the blood pressure usually fell to levels below 50 mm. Hg. Blood pressures, oxygen consumption, plasma protein and hemoglobin concentrations were determined on the dogs before and during the period of shock and also after the injection of the lyophile ascitic fluid.

Blood pressure and oxygen consumption which were low during shock regained normal values after the injection. Plasma protein values fell, as a result of dilution from the tissues, during the period of shock and fell further after the injection; this latter fall being due to the fact that the concentration of the protein in the fluid was less than that in the blood and also because the fluid was hypertonic with respect to salts. The plasma proteins returned rapidly and regained normal values in a few days.

On the whole the dogs survived the experience without detectable damage.

Development of the diurnal temperature and motility patterns in a baby.

F. J. MULLIN. University of Texas Medical School, Galveston, and University of Chicago, Ill.

This report concerns the study of body temperature and motility each month for the first fifteen months of life of only one subject. It shows the development of the diurnal cycle of body temperature and motility as a continuous transition from the condition present at birth. Heretofore this has been studied only in isolated samples at various time intervals.

The motility was measured by a kymograph attached to the bed springs. On the minimum more-or-less uniformly distributed activity present at birth there is superimposed the greater motility later present during the waking hours. There is the gradual development of a definite cycle of motility in relationship to the daytime nap periods and the long sleep at night of the fifteen month old baby boy. The actual time spent in motility was measured by a spirometer system which caused a clock to run only while the baby was moving.

The body temperature was recorded every two hours for from four to five successive days about once a month. This showed the development of a definite diurnal cycle spreading over a range of three degrees Fahrenheit by the eleventh month from a spread of about a degree at birth. As the temperature spread develops it manifests itself as a progressive lowering of the minimum temperature level at night with practically no change in the maximum temperature reached during the daytime. From the sixth month the temperature curve became progressively more regular with less and less varying distribution of the body temperature readings at corresponding times on successive days.

The recovery of electrical excitability in the mammalian ventricle. L. H. NAHUM and H. E. HOFF. Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

The electrical excitability of the cat's ventricle has been explored by

means of induction shocks applied to the exposed heart at various phases of the normal cardiac cycle. Three different types of recovery were found: 1. The absolutely refractory period was followed by a short relatively refractory period and this by a supernormal period which ended some time before the onset of the subsequent beat; this type of recovery was usually observed in animals anesthetized with sodium amytal. 2. In the second type, usually found in decerebrate cats with the common carotids ligated, the supernormal period was absent, and a relatively long period of level threshold occurred before the next systole. 3. In the third type, more often found in decerebrate cats with intact common carotids, but also in normal animals with light amytal anesthesia, the relatively refractory period was greatly prolonged, and the threshold declined progressively to the end of the cycle.

A supernormal period and the long relatively refractory period were usually found after the end of the T-wave as recorded from conventional leads. However, both semi-direct and monophasic leads indicated electrical activity continuing in the ventricle beyond the T-wave and until the next ventricular complex. Conventional leads also show at times a U-wave in the same part of the cycle as the supernormal period. The end of the T-wave does not therefore mark the end of electrical events in the cardiac cycle nor the termination of the recovery period. Supernormality is induced or augmented by increase in temperature and is reduced or abolished by cold and hyperventilation. Mecholyl in amounts sufficient to halve the normal frequency abolished the supernormal period but prolonged the relatively refractory period. Digitalis in doses sufficient to produce bundle-branch block and ventricular ectopic rhythms, does not cause supernormality in the ventricle.

| ANIMAL | PREP. | RECTAL TEMP. | DURATION IN SECONDS | | | |
|--------|----------------------------|--------------|---------------------|-----------|-------------|--------------------|
| | | | Length cycle | Abs. ref. | Relat. ref. | Supernormal period |
| | | °C. | | | | |
| 1 | Amytal | 37 | 0.34 | 0.18 | 0.19-0.22 | 0.22-0.28 |
| | Mecholyl | 37 | 0.71 | 0.30 | 0.31-0.57 | 0 |
| 2 | Amytal | 37 | 0.29 | 0.17 | 0.18-0.19 | 0.19-0.22 |
| | Cold | 26 | 0.68 | 0.43 | 0.44-0.54 | |
| 3 | Amytal | 36 | 0.29 | 0.19 | 0.20-0.27 | 0 |
| | Digitalis | 36 | 0.30 | 0.19 | 0.20-0.27 | 0 |
| 4 | Decerebrate, carotids tied | 36 | 0.29 | 0.18 | 0.19-0.23 | 0 |
| 5 | Decerebrate, carotids open | 36 | 0.33 | 0.22 | 0.22-0.32 | 0 |
| | Heat | 40 | 0.22 | 0.10 | 0.10-0.14 | 0.14-0.16 |

Gastro-intestinal hemorrhages in dogs from acetyl-choline and pitressin.

H. NECHELES and W. MASUR (by invitation). Departments of Gastro-Intestinal Research, Michael Reese Hospital, and of Physiology, University of Chicago, Ill.

Acetyl-choline and pitressin have been shown to reduce blood flow through the stomach. Pitressin was found and acetyl-choline is suspected to produce gastric ulcers. Acetyl-choline is liberated at the endings of the vagus nerves of the stomach and pitressin apparently is liberated by afferent stimulation of the vagi.

In order to test the effect of acetyl-choline and pitressin on the gastro-intestinal tract unanesthetized dogs were given constant intravenous

injections of 1, acetyl-choline; 2, acetyl-choline plus pitressin, 3, pitressin. Central vagus stimulation was employed in unanesthetized dogs to test the effects of possible reflex liberation of pressor principle from the posterior pituitary.

Twenty-two dogs were used. Six received constant injection of acetyl-choline. Most of these dogs showed a hemorrhagic condition of stomach, duodenum, and jejunum, as well as free blood in these organs and in the feces.

A second group of two dogs received acetyl-choline and pitressin and showed similar findings. In addition hemorrhagic condition and free bleeding was found to be present in the colon.

The third group of nine dogs received pitressin only. The outstanding finding in this group was hemorrhagic condition and bleeding in the colon. The condition of the upper gastro-intestinal canal was similar to that described in the first two groups, but to a lesser degree.

In the last group of dogs the right central vagus was stimulated intermittently. A medium degree of hemorrhagic condition was found to be present in the entire gastro-intestinal tract of most of the dogs.

Microscopically most of the specimen showed engorgement of the capillaries of the mucosa with focal hemorrhages. The picture was in contrast to the large amount of hemorrhage found in most of these dogs a number of whom actually bled to death.

Changes found in other organs as well as in the upper gastro-intestinal tract correspond to such described by previous authors.

Constant injection of acetyl-choline seems to have a toxic effect on the upper part and pitressin on the entire gastro-intestinal tract especially on the colon.

The influence of various factors on B-hydroxybutyric acid utilization. NORTON NELSON (by invitation), ISABELLE GRAYMAN (by invitation) and I. ARTHUR MIRSKY. The May Institute for Medical Research of the Jewish Hospital, and the Department of Biochemistry, University of Cincinnati, O.

The utilization of B-hydroxybutyric acids was determined in rats after the intravenous administration of a definite amount of sodium B-hydroxybutyrate and the subsequent determination of the total ketone body content of the whole rat. By this means it was possible to obtain data which permitted the estimation of the amount of ketone bodies utilized during a definite period of time. Our data demonstrate that there is no statistically significant difference in the utilization of B-hydroxybutyric acid by rats receiving food and carbohydrates as compared with rats fasted 24 hours and receiving saline. Identical conclusions are derived from data obtained from studies on nephrectomized and intact rats. This suggests that the decrease in ketone excretion consequent to glucose administration observed by others must be attributed to a diminution of spontaneous ketogenesis.

The influence of sex on ketone utilization was studied in several series of animals and this data indicate that there is no statistically significant difference between the utilization of ketones by male and female rats. Any difference in ketone excretion in the urine observed by others in the two sexes must be due to differences in spontaneous ketogenesis and not to differences in the rates of ketone utilization.

In view of the hypothesis that adrenalectomy results in an increase in ketone utilization, a series of adrenalectomized animals were studied. Our data demonstrate that 48 hours after adrenalectomy the utilization of ketone bodies is significantly decreased instead of being increased.

The effect of thyroidectomy upon lactation. WARREN O. NELSON. Department of Anatomy, Wayne University, College of Medicine, Detroit, Mich.

It has been shown by Nelson and Tobin (*Endocrinology* 5: 670, 1937) that thyroidectomy in the rat during pregnancy has no appreciable effect upon the rate of growth of the resultant litter during the immediately subsequent lactation period or during later lactation periods. Recently, Folley, (*J. Physiol.* 93: 401, 1938) has claimed that thyroidectomy performed during lactation resulted in a marked retardation of growth in suckling rats. Since his results are so clearly at variance with our own, it has seemed important to reinvestigate this problem.

Thyroidectomy was performed in 15 rats on the second or third day of lactation and in 17 on the 18th day of pregnancy. The effect of thyroid absence on the current or subsequent lactation was studied in all of these and, in addition, the effect on later lactations has been examined in 21 instances. The growth rates of 63 litters from normal mothers have served as control data.

All animals received the same diet and treatment with one exception. As in our earlier work, it was observed that during the latter part of pregnancy thyroidectomized animals are subject to tetany. To combat this, parathormone was administered routinely during the latter stages of pregnancy.

It has been possible to confirm our earlier observation that the growth rate of suckling rats is not impaired significantly by thyroidectomy. As an example, the following figures represent the average weight of young rats from the different groups at 21 days: Rats from normal mothers—35.3 grams, rats from mothers thyroidectomized during pregnancy—35.7 grams, rats from mothers thyroidectomized during lactation—31.8 grams, rats from mothers thyroidectomized during an earlier pregnancy or lactation—36.3 grams. From these data it is clear that lactation is not impaired in any of the groups with the exception of those operated during lactation. The slight retardation of growth observed in the young of these animals may, in part, explain Folley's findings.

Further studies in the metabolic, ketogenic and glycostatic effects of pituitary extracts. A. H. NEUFELD (by invitation) and J. B. COLLIP. Department of Biochemistry, McGill University, Montreal, Canada.

Extended studies on the effect of extract no. 622 and of other somewhat similar extracts upon the metabolic rate and the R.Q. of rabbits will be reported. This work represents an extension of that previously reported.

The ketogenic action of such extracts represents only a very small fraction of the total ketogenic activity present in saline or dilute alkaline extracts of fresh anterior lobe tissue. Very potent ketogenic fractions have been obtained, however, and a comparative study has been made of the relative strength of such preparations in regard to the effect upon liver fat in fasted mice (dba strain) as well as the ketogenic action in rats. It was found that these two properties tended to be associated even though

extracts differed widely as regards other properties such as growth and thyroid-stimulating effects (confirming Best and Campbell). Boiling at pH 7 for 10 minutes completely inactivated such preparations.

Extract no. 622 and its dialysate produced in mice (C57 strain) a marked decrease (15-50 per cent) in the total body fatty acids. No significant change was noted, however, in the total body glycogen.

Further experiments with rabbits treated with extracts rich in the specific metabolic principle confirmed and extended the glycostatic effect of such extracts previously reported.

Blood volume in shock. F. NEUWELT (introduced by H. Necheles). Departments of Gastro-Intestinal Research, Michael Reese Hospital, and of Physiology, University of Chicago, Ill.

The dye method of Keith, Rowntree and Geraghty or one of its modifications has been employed both experimentally and clinically to determine blood volume and is generally accepted to be reliable and fairly accurate. We have employed one of these modified methods to determine the blood volumes of 1, normal unanesthetized dogs; 2, dogs subjected to hemorrhage and resultant shock; 3, splenectomized dogs, and 4, dogs under the effects of various pharmacologic agents. Although the vital red dye method has certain inherent errors, we found that the results in normal dogs are quite reliable and that repeated determinations in the same animals revealed very similar values.

Dogs following splenectomy have a decreased circulating blood volume, the diminution being reflected in the plasma volume as well as the corpuscular elements. Such animals develop shock from hemorrhage more easily than normal dogs, and their blood reserves are greatly diminished.

Under conditions of controlled hemorrhage and resultant shock, we feel that the blood volume determinations become inaccurate and no longer serve as a reliable criterion for the total circulating blood volume of the dog.

Pharmacologic agents affecting the capillary bed cause marked changes in the blood volume determinations.

Blood volumes cannot be calculated from red blood cell count, hemoglobin, hematocrit, blood protein, etc. Since the dye method is unreliable in shock following hemorrhage, we have no accurate laboratory means at present to provide an index of degree or severity of this condition in our experimental animals.

Continuously recorded variations in the specific gravity of anesthetized dogs produced by various respiratory modifiers. HAYDEN C. NICHOLSON and ROBERT TRIMBY (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

At the 1938 meetings of this Society Henderson and Turner reported a study of "respiratory variations in the weight of a man submerged in water." Since the recording of respiration by changes in buoyancy seemed to offer some advantages over the more commonly used methods we have attempted to adapt it to give a continuous record.

The dog is placed on a light dog board suspended in a tank of isotonic saline. Mounted above the tank is a short lever moving on an axis consisting of a horizontal steel rod supported on two ball bearing mountings. A stiff flat steel spring passes vertically through the center of this axle. The dog board is suspended from this lever close to the fulcrum. The

length and thus the stiffness of the spring may be varied. On the opposite end of the lever is a screw which presses on a disc on a rubber tambour. This tambour is connected by rubber tubing with a smaller recording tambour the magnified movements of which are recorded on smoked paper. During normal breathing this record showing the varying specific gravity of the animal throughout the respiratory cycle is indistinguishable from the spirometer tracing over which it has the advantage that changes in the tonus of the respiratory muscles are shown independent of changes in oxygen consumption.

With this method it is found that the increased amplitude of respiration produced by carbon dioxide administration is due both to an increase in the depth of inspiration and to a more complete emptying of the lungs on expiration.

The stimulation of respiration by lack of oxygen is ordinarily associated with no increase in expiration, the extent of expiration in many cases being actually diminished.

In cyanide hyperpnea expiration may be more complete or it may be markedly diminished, the respiratory movements occurring at a very high inspiratory volume. The two effects may occur successively in a single administration.

Carbonate apnea may occur at the normal expiratory volume but usually somewhat above it.

Influence of ether narcosis on pH of cortex and electrocorticogram. L. F. NIMS (by invitation), W. S. McCULLOCH and J. G. DUSSER DE BARENNE. Laboratories of Neurophysiology and Physiology, Yale University School of Medicine, New Haven, Conn.

Ether narcosis results in a lowered pH, i.e., "more acid reaction" of the cortex of experimental animals. This change is closely paralleled by a diminution in the electrical activity of the cerebral cortex. The changes are completely reversible.

Circuit theory of nervous conduction. F. OFFNER (introduced by R. W. Gerard). Department of Physiology, University of Chicago, Ill.

Consideration of the effect in nerve of membrane permeability and polarizability on current flow, according to Lillie's depolarization theory of conduction, allows a mathematical treatment of nervous action in terms of an equivalent electrical transmission line containing resistance, leakance, and capacitance. The explicit assumption is made that the time factor of excitation is the R-C time constant of the membrane. It is further assumed that when the membrane potential falls below a critical value its permeability (leakance) suddenly increases. Then, under defined conditions, this results in a self-propagating impulse, the velocity of which is calculated. If the critical potential is below a certain minimum value, the equation shows sustained conduction to be impossible and predicts conduction with a decrement.

The assumptions made are consistent with the experimental results of Cole.

Electrical activity of the lateral geniculate nucleus of the cat. J. L. O'LEARY (by invitation) and G. H. BISHOP. Washington University School of Medicine, St. Louis, Mo.

The synaptic interruption of the optic pathway in the lateral geniculate offers the opportunity of obtaining, from pairs of electrodes suitably disposed, critical records of the activity of the elements involved. Such records demonstrate two waves in the optic tract, the faster of which is followed by a postsynaptic response in the radiation. The slower tract wave propagates to the superior colliculus. With stimuli sufficiently strong to excite the C fibers of other nerves, a large slow postsynaptic wave is recorded from the superior colliculus. Following the postsynaptic optic radiation response, slow potentials may occur. Commonly a spike is followed by a slow potential of the same polarity and rising from its falling phase, this followed by a still slower wave of opposite polarity. They do not increase with stimulus increase beyond the maximum of the first tract spike and are recorded from the radiation distant from the geniculate. Their durations are comparable to those of cortical waves. Other spike potentials, presumably assignable to internuncial neurones, are recorded only in the vicinity of the geniculate cell layers.

From any two electrodes in contact with tissue in which an active pathway is embedded potentials of all elements concerned in activity may be recorded, even though the electrodes are not in immediate contact with active tissue. If active tissue lies between electrodes, the nearer electrode is more negative. If one electrode is thrust *into* active tissue, the local injury will usually act as a killed end and this electrode becomes *positive* with activity. If the proximal electrode is near one end of an active tract a diphasic record is obtained, if near the middle a triphasic record. If the active region is limited in length the record is correspondingly monophasic. A synaptic ending gives a monophasic record from electrodes subtending it. In general, the form and polarity of the record from active tissue between two electrodes is a function of the direction of the activated pathway, of the distance from active tissue to electrodes, and of the length of the active elements.

The effects of heat on bile flow, a quantitative and qualitative study. S. L. OSBORNE and LEON GOLDMAN (introduced by F. T. Jung). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Acute experiments were made on forty large healthy dogs. The animals were placed on a standard diet. Under barbital anesthesia the common bile duct was cannulated, the gall bladder drained, and the cystic duct ligated. Drop flow was recorded by means of an electrical circuit and volume was measured. Following a suitable control period heat was applied using the inductotherm until 42°C. was reached.

The total amount of bile flowing during the entire experiment was determined and the bile analyzed for cholic acid, cholesterol, bile pigments and fatty acid concentrations.

It was found that heat as applied increased both the minute drop flow and the volume of bile. Cholic acid concentration decreased in both control and heated dogs but was more marked with the control animals. Cholesterol response was variable. Fatty acids increased. Biopsy studies of the liver were also made.

Cardiac action of sympathomimetic amines in cyclopropane, ether and chloroform anesthesia. O. S. ORTH (by invitation), J. W. STUTZMAN (by in-

vation) and W. J. MEEK. University of Wisconsin Medical School, Madison.

Dogs were maintained until equilibrated on mixtures of ether, chloroform, or cyclopropane diluted with oxygen and sufficient to produce deep surgical anesthesia. Various sympathomimetic amines (adrenalin, ephedrine, propadrin, benzedrine, cobefrin, kephrine, epinine, paredrine, arterenol, synephrin and neosynephrin) in doses equivalent in blood pressure raising power to 0.01 mgm/kgm. of adrenalin were diluted with Locke's to a standard volume and injected intravenously at a standard rate. Electrocardiograms (lead II throughout) of the arrhythmias resulting allowed the determination of the stimulating or sensitizing effect of each of the anesthetic agents on the autonomic tissues of the dog's heart.

Information regarding the active group in the molecule of the amine; the effect of premedication by some of the barbiturates; and evidence as to whether the cardiac effects under cyclopropane are due to the direct action of the agent or to an accompanying anoxemia, has been obtained.

Cyclopropane greatly enhances the activity of adrenalin on the autonomic tissues of the dog's heart. In controls adrenalin produces only escape phenomena but under the anesthetic multifocal ventricular tachycardia occurs in practically all experiments. Adrenalin is therefore contraindicated in cyclopropane anesthesia. Comparable pressor doses of arterenol, epinine, cobefrin, and kephrine acted similarly to adrenalin on the ventricular automatic tissue in cyclopropane anesthesia. Cyclopropane sensitizes the ventricular automatic tissue to those sympathomimetic amines with the catechol nucleus. Ephedrine, paredrine, benzedrine, propadrin, synephrin, and neosynephrin do not, in comparable pressor dosages, exert such effects in cyclopropane anesthesia. However, all but neosynephrin markedly accelerate the sino-aortic rate. In the dog under cyclopropane, neosynephrin is the sympathomimetic amine most favorable to the heart. Anoxemia was not a factor in the sensitization. Premedication with sodium barbital, amytal, or nembutal gave no protection against cardiac sensitization from cyclopropane.

In ether anesthesia neither ephedrine, arterenol, cobefrin, or neosynephrin produced ventricular tachycardia. Presumably then ether does not sensitize the heart to any of the common types of amines. All except neosynephrin increased the sino-aortic rate. Under chloroform some cardiac sensitization was shown to cobefrin and arterenol, less to ephedrine, and none to neosynephrin.

Electrodialysis of tissue chloride. ROBERT H. OSTER and WILLIAM R. AMBERSON. Department of Physiology, School of Medicine, University of Maryland, Baltimore.

A method of removing chloride from animal tissues prior to titration has been developed. It is based on the electrodialysis method of Joseph and Stadie for the simultaneous determination of Cl and total base in blood serum. The apparatus is designed so as to permit the use of fairly large pieces of tissue, cooling of tissue during electrodialysis, and rapid removal of the electrolysate at frequent intervals. The Cl is electrodialyzed through sheets of Du Pont cellophane into an anode chamber containing a solution of acetic acid and glucose. It is then titrated by Whitehorn's modification of Volhard's method. Determinations have been made on various tissues from normal dogs and from other animals

in which the Cl concentration has been diminished by several methods. The analytical results have been compared with values obtained by Van Slyke's wet-ashing method as modified by Wilson and Ball, and Eisenman.

For a given tissue the Cl values obtained by the two methods check within less than two per cent. Under identical current conditions the rate of removal of Cl from skeletal muscle is the same as from a block of agar gel containing Cl. Certain other tissues, especially brain, show significantly lower rates than the agar control. Brain tissue after aging for several days shows an increased rate of removal and after heating at 100°C. for one hour loses its Cl as rapidly as does the agar control. In most cases the rate of Cl removal is proportional to the Cl concentration within the tissue.

*Direct current potentials associated with human ovulation.*¹ RICHARD PARMENTER (introduced by H. S. Liddell). Department of Physiology, Cornell University Medical College, Ithaca, N. Y.

In the present study made upon eight healthy normal women, the potential difference between the index fingers was recorded daily over a period of several weeks. A vacuum tube microvoltmeter was used which was essentially similar to the one employed by Reboul, Davis and Friedgood in their studies of ovulation in the rabbit.

Contrary to the findings of Burr and Musselman no consistent unidirectional polarity of the right index finger was observed, the potential difference of this member fluctuating both in magnitude and polarity from day to day.

In seven of the eight women forming the subject of this study a very marked rise in potential difference was observed to occur at some moment between the ninth and sixteenth days following the onset of the preceding menses. This "ovulation potential" reversed in sign from the first recording to the second in three of the cases studied. The magnitudes of the potentials observed agree reasonably with those reported by Rock, Reboul and Snodgrass, being very conspicuously above the normal day to day variations.

Observations on four of these women are being continued.

A comparison of the responses to continuous currents of excised nerve and nerve with a blood supply. HORACE O. PARRACK (introduced by Kenneth S. Cole). Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

The theory of nerve excitation proposed by A. V. Hill takes into account "accommodation," the process by which nerve adjusts itself to a continuous current. Hill and his co-workers have devised methods for measuring and have determined the time constant of "accommodation" in excised frog's nerve.

That the magnitude of "accommodation," as well as its time constant, may be of importance in nerve function is at once apparent. The present problem deals with the determination of the magnitude of "accommodation" in excised nerve, and in nerve with an adequate blood supply. The sciatic nerve of the frog, *Rana pipiens*, has been used. The half maximal action potential of the alpha fibers has served as the index of nerve ac-

¹ Aided by a grant from the Josiah Macy, Jr. Foundation.

tivity. All experiments have been performed at room temperatures of 22° and 24°C.

The following conclusions can be drawn: In nerves possessing a circulation "accommodation" is less than in excised nerve and appears later.

The magnitude of "accommodation" has regularly been increased by Ca^{++} ions in excised nerve. The effects of Ca^{++} ions on nerve possessing a blood supply is under investigation.

The effect of K^{+} ions on the magnitude of "accommodation" is uncertain, but may increase it.

Both Ca^{++} and K^{+} ions cause the "accommodation" to start earlier.

The magnitude of "accommodation" is usually less at the anode than at the cathode and less regular in its course.

The course of the irritability changes occurring on the break of a continuous current have been followed for currents of short and long durations. The changes at the cathode are essentially the same for long or short durations and weak or strong currents. The changes at the anode with long durations differ markedly from those at short durations, particularly when the current is strong. The anodal depression persists for a relatively long time following long strong currents.

The effect of yeast extract on active intestinal absorption of chloride. H. C. PETERS (introduced by A. G. Mulder). Department of Physiology, University of Tennessee, Memphis. (Read by title.)

A yeast extract was prepared by shaking 2.5 grams of dry yeast with 100 cc. of a solution containing 0.5 isotonic sodium sulfate and 0.5 isotonic sodium chloride and filtering. A control solution was prepared without yeast. Absorption of chloride from these solutions was studied in adjacent ileal loops in anesthetized dogs.

Yeast extract increased chloride absorption 20-45 per cent. It seems likely that this effect is due to the stimulating influence of yeast extract on villous activity (Verzar and Kokas. *Pflüger's Arch.* 217: 397, 1927). No effect on net water absorption was found.

*A new method for the gradual occlusion of coronary arteries.*¹ K. R. PHELPS (introduced by C. S. Beck). Department of Surgery, Western Reserve University, Cleveland, O.

The experimental studies for augmenting the blood supply to the myocardium by operation, have given rise to a number of methods for producing coronary occlusion. One requirement of this work is a method for occluding the artery gradually in order to simulate the occlusion produced by sclerosis. Various methods have been used in this work by Beck, Tichy, Mautz, Thornton and Robertson. Each of these methods required multiple surgical procedures at which time the additional constriction was abruptly produced. Up to the present time, there is in existence no technique whereby an artery can be occluded gradually and completely at a fixed rate.

The clamp to be here described utilizes the principle of osmosis. It is constructed of silver, cylindrical in form, approximately 2.5 cm. in length over all, with an inner hollow piston which, before use, is filled with a hypertonic solution of sucrose, sealed in by means of a semi-permeable

¹ Aided by a grant from the Josiah Macy, Jr. Foundation.

collodion membrane. An L-shaped extension at the other end of the clamp is passed around the artery to be occluded, and the open side of the L is then closed by means of a sliding arm. The rate of dialysis of tissue juices through the membrane to increase the volume of the contained charge, and thus push down the piston to produce constriction of the artery, can be varied within wide limits by varying the membrane porosity. Occlusion of all major coronary arteries has been performed, on different dogs, in this laboratory, under electrocardiographic and roentgenologic control.

This method of occlusion enjoys the following advantages: 1, requires one surgical procedure; 2, constriction proceeds progressively at a constant rate, and 3, the rate of constriction can be controlled by selection of membranes of desired porosity.

Studies of skin temperature responses following adrenaline. R. A. PHILLIPS, J. C. HINSEY and J. D. HARDY (by invitation). Russell Sage Institute of Pathology in affiliation with the New York Hospital, and Departments of Physiology and Medicine, Cornell University Medical College, New York City.

Observations of skin temperatures of the foot pads were made on un-anesthetized and anesthetized (Nembutal intravenously) cats given 10 gamma to 200 gamma of adrenaline intravenously. The temperatures were observed radiometrically in a constant temperature room. Cooling curves were obtained from the foot pads after complete occlusion of the blood supply to the limbs by section of the abdominal aorta and inferior vena cava.

The following formula was empirically developed from several experiments to relate the temperature of the pad (T_t), to the time after occlusion (t), to the room temperature (T_o) and to the initial pad temperature (T_s): $T_t = T_s - (.437 \log (1+t)) (T_s - T_o)$.

The theoretical cooling curve coincided with the observed cooling curves in complete occlusion within the allowable experimental error. The temperature changes in the foot pads of other experimental animals was compared to that for complete occlusion of blood flow on the assumption that any observed difference would be due to difference in heat brought to the surface of the pad by the blood stream.

With anesthesia the responses to adrenaline of the normal and post-ganglionic sympathectomized extremities were identical up to a certain point. Thereafter, the temperature of the normal pad rose rapidly while that of the sympathectomized extremity rose more slowly. With large doses of adrenaline the sympathectomized extremity continued to fall to a lower level than did the normal and then slowly returned.

The responses to the slow infusion of adrenaline were observed on one patient with Raynaud's Disease before and after thoracic sympathectomy. There were identical decreases in skin temperatures of the back of the fingers before and after sympathectomy, but following sympathectomy the return to the initial level after adrenaline was greatly delayed.

It should be stressed that it is extremely important to know the body temperature, room temperature as well as the skin temperature in using skin temperature responses as an indication of vasomotor activity. Of equal importance is the degree of relaxation of the observed vessels.

*The maturation of explanted human ovarian ova.*¹ GREGORY PINCUS.

Biological Laboratories, Clark University, Worcester, Mass.

From a set of human ovaries excised at hysterectomy ova were obtained by flushing out the visible follicles. All obviously atretic ova were discarded. 144 ova were treated as described in the following table, fixed, sectioned, and stained for microscopic examination. The eggs cultured were in human serum.

| TREATMENT | HOURS CUL- TURED | TOTAL NUM- BER OF EGGS | NUM- BER OF EGGS WITH SPIN- DLES | NUM- BER OF EGGS WITH POLAR BODIES | PER CENT OF EGGS ACTI- VATED |
|---------------------------------------|------------------------|---------------------------------|---|---|--|
| None | 0 | 34 | 0 | 0 | 0 |
| None | 8½ | 4 | 1 | 1 | 50 |
| None | 17-24 | 13 | 1 | 4 | 38 |
| Sperm extract 15 to 30 mins. | ½ | 5 | 0 | 0 | 0 |
| Sperm extract 15 to 30 mins. | 1-2 | 10 | 3 | 0 | 30 |
| Sperm extract 15 to 30 mins. | 15-48 | 40 | 8 | 10 | 45 |
| Sperm extract 15 to 30 mins. | 24-72 | 9 | 1 | 2 | 33 |
| then to rabbit fallopian tubes | | | | | |
| Heated to 46°C for 3 mins. | 2 | 8 | 2 | 1 | 38 |
| Heated to 46°C for 3 mins. | 24-48 | 5 | 0 | 2 | 40 |
| Hypertonic solution 5 to 6 mins. | 24-48 | 16 | 0 | 7 | 44 |

The data of the table demonstrate that: 1, ova as taken from the follicles are rarely in maturation division (cf. Allen, Pratt, Newell, and Bland, *Am. J. Anat.* **46**: 1, 1930); 2, explantation without preliminary treatment initiates maturation in about 40 per cent of the ova; 3, explantation after preliminary treatment with sperm extracts, brief exposure to supranormal temperature or to hypertonic solution does not appreciably increase the per cent activated; 4, the transplantation of treated eggs to the rabbit fallopian tube does not increase the per cent activated; 5, the maturation division must ordinarily begin by one to two hours after explantation. Most of the ova that did not enter maturation were probably in early stages of atresia.

The localization of the respiratory center in the cat. R. F. PITTS, H. W. MAGOUN and S. W. RANSON. Institute of Neurology, Northwestern University Medical School, Chicago, Ill.

In a study of the respiratory responses obtained from stimulation of the brain stem of the cat utilizing the Horsley-Clarke technique we have noted the striking character and definite localization of two types of response. One of these, a tonically maintained deep inspiratory apnea which appears to be in maximal inspiration, is localized to the ventral reticular formation of the medulla immediately overlying the cephalic four-fifths of the inferior olive extending some 3 mm. to either side of the midline. It thus roughly extends from 2 mm. caudal to the acoustic tubercle to 1 mm. caudal to the obex. The other response which is a maintained expiratory apnea, in some instances in maximal expiration, is localized

¹ The writer is indebted to Doctors Pemberton, Smith, and Rock of the Free Hospital for Women, Brookline, Mass. for providing the specimens used in these experiments.

to the dorsal reticular formation of the medulla, dorsal to, slightly cephalic to, and cupped over the cephalic end of the inspiratory reticular formation.

The following lines of evidence are offered that these two localized reticular regions constitute the inspiratory and expiratory divisions of the respiratory center. The respiratory responses are well localized within the region which previous workers have vaguely defined as the respiratory center. Responses are coordinated respiratory acts involving both thorax and diaphragm. The responses are probably not due to stimulation of afferent or efferent fiber tracts but to stimulation of a neurone field closely interrelated synaptically. They are constant responses only quantitatively influenced by strength and frequency of stimulation. They are independent of the anaesthetic used and may be obtained in unanaesthetized animals with electrodes sealed in the skull. Inspiratory apnea may be maintained till death of the animal in from 3 to 6 minutes. Coordinate respiration may be attained by rhythmic stimulation of these centers.

Pseudo-hypophysectomy produced by inanition. LEO POMERANTZ (by invitation) and MICHAEL G. MULINOS. Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York City.

Rats fed with insufficient amounts of a well balanced diet became anestrus when the body weight loss exceeded 10 per cent. (Proc. Soc. Exper. Biol. and Med. **40**: 79, 1939.) Refeeding and consequent gain in body weight caused the cycles to return to normal. Two to four R. U. of estrogen or pregnancy urine extract injected into acutely or chronically starved rats caused them to estruate. Starvation did not prevent the responsiveness of the gonads and genitalia to these substances. Implantation into rats with inanition anestrus, of pituitary glands of either normal or acutely starved rats, resulted in estruation of about equal strength and duration.

The ovaries of chronically starved rats weighed less than the controls and there was a decrease in the number of large follicles. The ovary became filled with a cell type which was poor in cytoplasm and contained a nucleus similar to that of a plasma cell. This cell resembled the "wheel cell" described by Selye et al. (Endocrinology **17**: 494, 1933) which they regarded as a "typical deficiency reaction of the ovary after hypophysectomy."

This functional pseudo-hypophysectomy produced by inanition was reflected in other endocrine glands. The testicular and adnexal tissues of male rats were also affected.

Supporting, timing, and recording apparatus. F. H. PRATT and W. T. PORTER. Boston University School of Medicine, Boston, and Dover, Mass. (Demonstration.)

Mounting rods and special clamps. Improved magnetic-repulsion interrupter. Adjustable signal magnet with vibrator tuned for alternating current and containing a relay contact for separate circuit, make-shock cut-out, or drop recorder. Extra-light recording lever of balsa-wood, with sliding adjustment and counterpoise. Kymograph paper prepared with latex dry adhesive. Mendenhall pleural cannula. New Gaskell clamp.

The vitamin C requirement of man as determined by a prolonged study of the daily excretion and plasma concentration of vitamin C. ELAINE P. RALLI, GERALD J. FRIEDMAN (by invitation) and SOL SHERRY (by invitation). Department of Medicine, New York University College of Medicine, and the Third (New York University) Medical Division of Bellevue Hospital, New York City.

The vitamin C requirement was determined on 3 adult normal males. The patients were in the hospital during the entire period of the study on diets containing about 5 mgm. of vitamin C daily. The 24-hour urinary excretion of vitamin C was determined daily, the urines being acidified when voided and kept in dark bottles in the ice-box. The plasma concentration was determined 3 times weekly. The cases were observed for 110, 240, and 127 days respectively. After a period on the diet vitamin C was fed as cevitamic acid (Merck) beginning with 50 mgm. daily and increasing it to 75, then 100, 150, 200 and 350 mgm. It was found, in all 3 cases, that the maximum retention of vitamin C was effected on a daily dose of 100 mgm. Feeding more than this daily was accompanied by a prompt rise in the urinary excretion of vitamin C. Also on the daily intake of 100 mgm. the plasma level rose from whatever its initial concentration to 1 mgm. per cent or more. This plasma concentration once attained could not be maintained on 50 mgm. of cevitamic acid daily. These results suggest that 100 mgm. of vitamin C daily is the optimum daily dose for normal adults.

Vitamin C was determined in urine and plasma by the indophenol titration method in the first case and in the photoelectric colorimeter in the second and third cases. In case 2 comparative determinations were done by both methods. The titrimetric method gave urinary C values which were consistently higher by about 10 mgm. per day due to the interference of a certain amount of non-vitamin C reducing substances. As the determinations in the photoelectric colorimeter are extrapolated to zero time the error due to the interfering substances is reduced.

The length-tension diagrams of single muscle fibers of the frog. ROBERT W. RAMSEY and SIBYL F. STREET (introduced by H. A. Blair). School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

The magnified deflections of a quartz lever to which an isolated fiber was attached were recorded photographically. The tension at each length was obtained from the maximum of a maintained tetanus. Resting length (R.L.) was taken as the length at which maximum tension was developed. The mean (13 experiments) maximum tension = 3800 grams/cm². If the R.L. is taken as 100 per cent, the range of length over which contractions are normally reversible is from 66 per cent to 200 per cent. In terms of the maximum tension developed, the resting tension which may be small (3 per cent at 150 per cent R.L.; 10-20 per cent at 180-200 per cent R.L.) and is very variable seems to be due to adhering connective tissue and the sarcolemma. Consequently, the "length-tension diagram" is almost entirely due to tension developed. Between R.L. and 200 per cent R.L. the tension falls from a maximum to zero, along an approximately straight line. From a length equal to 66 per cent R.L. to R.L. the tension rises from 20-30 per cent to the maximum, at first rapidly and then more slowly. The total area under the length tension curve from 66 per cent R.L. to 200 per cent R.L. is approximately equal to $\frac{3}{4} \times \text{maximum tension} \times \text{resting length}$. If the fiber is allowed to shorten below 66 per cent R.L. an irreversible change sets in. With prolonged stimulation the fiber may be shortened to 20 per cent R.L.

The characteristic of this phenomenon is that upon cessation of the stimulus the fiber remains shortened and does not relax. In this range (66 per cent R.L. to 20 per cent R.L.) the tension falls from 20-30 per cent to zero. After the fiber is completely shortened a new length-tension diagram may be obtained over a range from 20 per cent R.L. to 200 per cent R.L. with a maximum tension equal to 50 per cent of the original maximum tension. The shape of this new length-tension diagram differs from the original, approximating to a rectangular hyperbola with a new "resting length." Within limits the fiber is reversible over the new diagram.

The functional measurement of the number of active glomeruli and tubules in the kidneys of normal and hypertensive subjects. H. A. RANGES (by invitation), H. CHASIS (by invitation), W. GOLDRING (by invitation) and H. W. SMITH. Departments of Physiology and Medicine, New York University College of Medicine and the Third (New York University) Division of Bellevue Hospital, New York City.

In 1924 Richards and Schmidt (Am. J. Physiol. **71**: 178, 1924) noted by direct microscopic observation that only a certain proportion of the glomeruli, or of the capillaries in individual glomeruli, were active at any one moment. This observation has been repeatedly confirmed, and it has been inferred from the behavior of glomeruli in the frog's kidney that there is similar intermittency of glomerular activity in the human kidney. Did such intermittency exist it would acquire increased significance in view of the possibility of a direct blood supply to the tubules in the human kidney via arterial-venous anastomoses (Spanner. *Ergänzungsheft z. Anat. Anz.* **85**: 81, 1938). The physiological importance of this point, and the clinical importance of determining whether there exists any tissue in the hypertensive kidney which is rendered ischemic by reversible vasoconstriction of renal arterioles, has prompted us to measure the quantity of active glomerular and tubular tissue by saturation methods.

The total active tubular tissue in the kidney has been measured by elevating the plasma level of diodrast to a point where all the tissue receiving blood is excreting this substance at the maximal rate (diodrast-T_m, as described by Smith, Goldring and Chasis. *J. Clin. Invest.* **17**: 263, 1938). The total active glomerular tissue has been measured by raising the plasma glucose to a point where the tubules are reabsorbing this substance at the maximal rate (glucose-T_m as described by Shannon and Fisher. *Am. J. Physiol.* **122**: 765, 1938). If any glomeruli can be opened or closed by physiological means the fact will be revealed by an increase or decrease in the number of nephrons reabsorbing glucose, and therefore by a corresponding change in the value of glucose-T_m. At appropriate plasma levels of diodrast and glucose these measurements reveal the quantity of functioning tissue, and are independent of variations in renal blood flow or filtration rate.

Diodrast-T_m and glucose-T_m have been measured in normal and hypertensive subjects under basal conditions, during renal ischemia induced by adrenin, neosynephrin, tyramine, etc., and during sustained renal hyperemia. The observations will be reported in detail.

The in vitro effect of thyroglobulin and thyroxin on tissue metabolism. DAVID RAPPORT and ATTILIO CANZANELLI. Department of Physiology, Tufts College Medical School, Boston, Mass. (Read by title.)

Thyroglobulin or thyroxin was added to the medium bathing various

guinea pig tissues, and the O_2 consumption was observed, using a Barcroft-Warburg differential manometer system. The tissues were sliced, except in the case of testis, when they were teased apart.

Thyroglobulin elevated the O_2 consumption of heart in CO_2 -free horse serum, the average rise being 22 per cent. In a mixture of 0.9 per cent NaCl, Sorensen PO_4 buffer (m75 or m150) and 0.1 per cent glucose, thyroglobulin increased the O_2 consumption of heart an average of 60 per cent. The control QO_2 's were lower in the salt mixture than in serum. Thyroxin had no effect on the O_2 consumption in either serum or salt.

In the case of kidney, thyroglobulin raised the O_2 consumption consistently in serum from a control QO_2 of 20. At the same levels of metabolism in salt, or in a Ringer's- PO_4 -glucose solution, thyroglobulin was no longer effective. In most cases the control QO_2 was definitely lower in salt or Ringer's than in serum, and then a stimulating effect of thyroglobulin appeared. Thyroxin had practically no stimulating effect on kidney in serum, and there was evidence of a depressive effect in salt and Ringer's.

The effect of thyroglobulin on testis was equivocal in all media. In serum there was a consistently greater O_2 consumption than in the controls, but almost within the limit of error. In salt and Ringer's, a stimulating effect appeared at low levels of metabolism. Thyroxin in serum had no effect, and, as with kidney, evidence of depression was seen in salt and Ringer's.

In salt, we duplicated previously published experiments on liver. In serum, however, thyroglobulin had little effect, except where the control QO_2 was relatively low. Thyroxin had no effect whatever in either medium.

There appeared to be in all tissues a "ceiling" above which thyroglobulin could not further elevate metabolism, and this "ceiling" was higher in serum than in either salt or Ringer's.

A method of establishing raised or lowered blood pH levels for a considerable period in rats. RUTH A. RAWSON and M. MASON GUEST (introduced by E. L. Scott). Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

In physiological problems it is often desirable to study the effects of blood pH changes. A simple method for raising or lowering the blood pH and maintaining it at the new level for several hours has been quantitatively studied.

Male rats, 100 days old, were used under the conditions stated in a concurrent paper. Two intraperitoneal injections of 5 cc. each were given 1 hour apart of isotonic solutions of NaCl, NH_4Cl or $NaHCO_3$. The animals were anesthetized with nembutal (0.70 mgm. per kgm. body weight) at various intervals up to eight hours following the last injection. A single sample of blood was drawn from the heart into an airtight, oxalated syringe which was equipped with a special needle designed to waste the first few drops of blood. The pH determinations were made, immediately following the bleeding, on whole blood at $38^\circ C$. using a modified McInnes and Belcher glass electrode and a Leeds and Northrup Universal pH potentiometer.

The average pH values (ca 15 animals) 1 hour after the last injection were with NH_4Cl 7.19 ± 0.07 , NaCl 7.30 ± 0.02 and with $NaHCO_3$

7.40 ± 0.06 . From work in progress it appears that the pH curve remains flat for 6 to 7 hours following the 2nd injection.

Factors influencing the activation of the enzyme, tyrosinase. O. M. RAY (by invitation), T. H. ALLEN (by invitation) and J. H. BODINE. Zoological Laboratory, State University of Iowa, Iowa City. (Read by title.)

The eggs of the grasshopper, *Melanoplus differentialis*, served as a source of tyrosinase and were ground up in water, buffered or unbuffered, isotonic salt solution. The solution of inactive tyrosinase was dialyzed at 0°C. in cellophane osmosis membrane. The amount of active enzyme is proportional to the oxygen utilized in oxidation of the substrate (tyramine-HCl). This was determined manometrically by the standard Warburg apparatus. The total amount of tyrosinase present (active and inactive) may be determined by the addition of 0.1 cc. 5 per cent Na oleate solution which gives maximum activation.

It was found that as dialysis progressed from 2 to 48 hours the activity of the tyrosinase increased proportionally. The change in the slope of the curves indicates the reaction changes from truly autocatalytic to catalytic, uni-molecular type and suggests the possibility of two enzymes.

As dialysis proceeds the activation of tyrosinase is accompanied by a marked reduction in solubility.

A striking characteristic of the action of tyrosinase activated by dialysis is that it can utilize only about 50 per cent of the available substrate due to the apparent formation of inhibitory substances during the course of the reaction. The addition of more substrate produces no further activity of the tyrosinase. However, the addition of Na oleate causes an immediate activation and the remainder of the substrate may be recovered. Activity produced by sodium oleate on inhibited tyrosinase is comparable to that produced in salt solution (enzyme inactive) and is less than the activity when the sodium oleate is added previous to inhibition by either means. It appears that these two types of inactivation are similar and that sodium oleate cannot entirely reactivate the enzyme. However, the presence of sodium oleate with the dialyzed enzyme either prevents the formation or the action of the inhibitory substances otherwise formed.

The inhibitory substances are both in solution and attached to the enzyme. The portion in solution inhibits fresh tyrosinase, is diffusible and is still present after the reaction has gone to completion by autooxidation. The portion attached to the tyrosinase cannot be removed by washing or dialysis and markedly decreases the solubility of the enzyme in salt solutions.

The rôle of the pituitary in the calorogenic action of vitamin D. C. I. REED, A. J. BARTOLI (by invitation) and J. FELDMAN (by invitation). Department of Physiology, College of Medicine, University of Illinois, Chicago.

It was shown previously that vitamin D does not produce any significant calorogenic effect in thyroparathyroidectomized dogs. In 8 hypophysectomized dogs with intact thyroids, 38,000 units of vitamin D per kilo per day produced no calorogenic effects except when subsequent microscopic examination showed remnants of the anterior lobe persisting. This dose invariably produced a calorogenic response in normal dogs, with

severe toxicity and weight loss. In these experiments vitamin D as viosterol usually produced some weight loss but no other evidence of toxicity except slight anorexia. Vitamin D in an electrically activated form produced no significant weight loss nor other evidence of toxicity.

Irritation of the upper respiratory tract and esophagus and its reflex effect upon the heart as shown by the electrocardiograph. L. CORSAN REID and DONALD E. BRACE (introduced by O. M. Cope). New York Medical College, New York City.

It has been recognized for some time that during anaesthesia, particularly in the lighter planes, and especially with the intratracheal technique, and probably most frequently with cyclopropane, that there arises abruptly, dramatically, a marked respiratory difficulty with very obvious evidences of circulatory derangement, and then after a variable period of time would as suddenly vanish leaving no clue as to its causal genesis or the mechanism of its disappearance.

On becoming acquainted with the work of Scherf et al. on pulmonary, coronary reflexes in lung emboli, the idea occurred to the authors that this above described and puzzling respiratory, circulatory syndrome might be adequately explained on the basis that the respiratory tract and esophagus were potential foci where irritative stimuli of any kind might set up vagal reflexes and cause marked inhibition and derangement of cardiac activity, and this altered cardiac activity constitutes the fundamental basis upon which rests all the above described findings.

It is shown in the demonstration of electrocardiographic tracings on operative cases with various anaesthetic agents, tube insertions, esophagoscopies, especially in light anesthesia with uniform preoperative medication of hyoscine and morphia that the above described mechanism is the actual explanation of the observed phenomena.

Therefore, this study shows that the introduction of mechanical devices or other irritative mechanisms into the upper respiratory tract or esophagus, especially in light anesthesia, is not without danger, and all such contemplated procedures should have adequate preoperative therapy to minimize vagal reflexes.

*Peripheral vascular action of oestrin in the rabbit and human.*¹ SAMUEL R. M. REYNOLDS and FRANCES I. FOSTER (by invitation). Department of Physiology, Long Island College of Medicine, N. Y.

Oestrin causes vasodilatation in various parts of the peripheral circulation, outside the reproductive tract. Examples of this are seen in vascular changes in the sex skin of certain sub-human primates and in the nasal mucosa of many species, including the human. In this work, direct observation is made of the vessels in the transilluminated ear of the rabbit, along with records of rectal temperature, room temperature and temperature of the skin of the ear before and after injection of oestrogens from natural and synthetic sources. The rabbits were ovariectomized. Observations have been made on rabbits with the ear intact, with the superior cervical ganglion excised and after administration of atropine and certain other drugs. Oestrin regularly causes dilatation of the pre-capillary

¹ Supported by grants from the Committee on Endocrinology, National Research Council and the Committee on Scientific Research, American Medical Association.

arterioles and venules having a diameter of 15 micra or less. In addition, the ground color of the ear becomes pinkish,—a sign of capillary dilatation (Grant. J. Clin. Sci. 2: 1, 1935-36). Under rigorous experimental conditions the skin temperature decreases steadily during the first 10-40 minutes from about 31-33°C. to one or two degrees above room temperature (down to 19°C.). In other experiments, oestrin did not cause any change in mean arterial blood pressure, an observation in line with clinical and experimental data. Over fifteen observations have been made on the effect of oestrin given intramuscularly on finger volume and skin temperature in the human male. Oestrin brings about an increase in finger volume of *circa* 0.3-0.5 cc. with no rise or a slight fall in skin temperature. The latent period for these changes is the same in both rabbits and the human: onset in 3-5 minutes, maximum 20-30 minutes. The effect persists during the period of observation (up to 2 hours).

The capsular fluid of Amblystoma punctatum eggs. OSCAR W. RICHARDS.
Research Biologist, Spencer Lens Co., Buffalo, N. Y.

Eggs are removed from the surrounding capsule for embryological experiments and placed into a suitable fluid for development. The capsular fluid was carefully collected from eggs at Harrison Stages 18-22 and 28-32 and analyzed for Na, Ca, K, Cl, and total protein content. The following physical properties were also determined: specific gravity, surface tension, osmotic pressure, bound water and viscosity. Comparison with Ringer and Holtfreter solutions shows that these substitutes for the capsular fluid are hypertonic. They also lack the protein content of the natural fluid. The fluid at these two stages will be compared. From this data solutions may be made which more nearly resemble the natural capsular fluid for the development of eggs following the operative procedures of the experimental embryologists.

Further observations on the carbohydrate, fat and protein appetite of vitamin B deficient rats. CURT P. RICHTER and BRUNO BARELARE (by invitation).

Psychobiological Laboratory, Henry Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md.

Results of previous self-selection experiments showed that rats deficient in the entire vitamin B complex have an aversion to carbohydrate and protein, and a craving for fat (Richter, Holt, Barelare and Hawkes, 1938). As the diet of the animals lacked all components of the vitamin B complex we could not determine on which components those changes in appetite depended.

The present studies were made with the same self-selection technic employed in earlier experiments. The following dietary assortment was offered in the separate containers: casein (autoclaved and purified), sucrose, dried baker's yeast, olive oil, cod liver oil, sodium chloride (3 per cent solution), potassium chloride (1 per cent), calcium lactate (2.4 per cent), dibasic sodium phosphate (8 per cent), magnesium chloride (0.5 per cent), and water. This technic greatly facilitates the production of a deficiency as each substance is purified, or nearly purified, and can be eliminated from the diet simply by removing the container. In these studies, for example, the animals were rendered vitamin B deficient by removing the yeast container.

Four separate groups of rats, offered the above assortment minus yeast,

were given access to one of the vitamin B components available in crystalline, or at least, highly purified form, thiamine, riboflavin (Merck), nicotinic acid or W factor (Elvehjem).

The group that had access to thiamine no longer showed the aversion to carbohydrate, but continued to show an aversion to protein. The riboflavin group showed no aversion to carbohydrate, but overcame at least in part the aversion to protein. The nicotinic acid and W factor groups gave less constant results. Thus, according to the rat's appetite, thiamine is most concerned with the regulation of carbohydrate utilization and riboflavin with the regulation of protein utilization.

With this study of the separate vitamin B factors we found, in addition, that the atrophy of the reproductive tract of vitamin B deficient rats depends largely, if not entirely, on the riboflavin intake and indirectly on the level of protein intake.

The S wave of the electrocardiogram. JANE SANDS ROBB and ROBERT C. ROBB (by invitation). Department of Pharmacology, College of Medicine, Syracuse University, Syracuse, N. Y.

Relatively little attention has been paid either to the duration or contour of the S wave, though its amplitude has been measured for calculation of axis deviation. Generally the QRS is considered as a unit. The interval from the end of S to the beginning of T has been studied in coronary disease. We have observed that the S-T interval may be normal in lesions of the superficial bulbo-spiral muscle but that the S may be slowed and this slowing may coincide with an R-T shoulder in another lead. An electrocardiogram showing prolongation of the QRS is presented. From the beginning of Q to the point where R crosses the isoelectric line is 0.06 sec. The duration of the S wave is 0.08 sec., that is, the prolongation of the QRS interval is entirely due to a change in the S. Among records of normal students many variations of S were observed. Sometimes the downstroke, the apex, and the upstroke are all quick (thin) lines. More often there is slowing either involving the apex to make it rounded, or the upstroke to make it thick. Occasionally the S is prolonged at its apex so that a horizontal line lasting up to 0.03 sec. results. When this occurs and the electrocardiogram is written on rapidly moving paper the contour is like that of a coronary S-T except that the duration is shorter. It would be premature to consider whether these S changes have pathological significance. Interference of auricular T waves, differentially slowed conduction in some area, possibly a scar, or even an anomalous Purkinje distribution could be causative. The "coronary" type of S change cannot be due to drugs in normal students and presumably is not due to injury currents. Since it occurs more frequently in lead 4R than in any other lead studied to date it behooves one to be very cautious in the interpretation of this lead.

The distribution of the Purkinje material in the septum of the beef heart.

JANE SANDS ROBB. Department of Pharmacology, College of Medicine, Syracuse University, Syracuse, N. Y. (Demonstration.)

Many have demonstrated Purkinje Material by means of ink injections of the sheath. Robb, Greene and Robb have demonstrated to this society the three dimensional distribution in ventricular walls which had first been injected and then cleared. A similar preparation of the septum of

the beef heart is now available. The anterior descending branch of the coronary artery and some of its branches are visualized. They show *no trace* of the ink. One may stress that neither this artery nor the interventricular groove in which it generally lies is an accurate indication of the position of the septum which may lie as far as $\frac{3}{4}$ inch to one side of the interventricular groove (also noted by Raessler). Since the Purkinje sheaths cannot be injected in man or the dog, most of our knowledge of this system comes from studies of pig, sheep, calf or beef hearts. In the beef heart a generous amount of ink is found in the anterior part of the septum. Such specimens do not support the concept that there is delay of conduction across the anterior interventricular septum due to lack of pathways.

Enzymatic hydrolysis of mucins by a bacterial enzyme and by vitamin C.

WILLIAM V. B. ROBERTSON and MARIAN W. ROPES (by invitation) and WALTER BAUER. Massachusetts General Hospital, Boston.

Few reports concerning the enzymatic hydrolysis of mucins or of their prosthetic polysaccharides have appeared. Neuberg and colleagues studied a bacterial enzyme which hydrolyzed chondroitin; Meyer, Smyth and Dubos reported the hydrolysis by the "autolytic enzyme" of pneumococcus of hyaluronic acid from vitreous humor, umbilical cord, synovial fluid and hemolytic streptococci.

We have isolated from cultures of an anaerobic organism an enzyme (mucinase) differing in certain characteristics from the "autolytic enzyme" of pneumococcus. Mucinase causes loss of viscosity and subsequent liberation of reducing substances and amino sugar from synovial fluid mucin and its prosthetic polysaccharide. It causes also loss of viscosity of the mucin from vitreous humor, umbilical cord and connective tissue.

Similar enzymatic activity was found in vitreous humor from normal cattle. This proved to be due to vitamin C. Reduced ascorbic acid is inactive but is activated by hydrogen peroxide or traces of copper. Excess of hydrogen peroxide causes decreased activity. Oxidation by ferricyanide, iodine or quinone causes no activation. However, vitamin C can be activated by hydrogen peroxide even in the presence of these substances.

Vitamin C and mucinase differ in specificity in that ascorbic acid causes rapid loss of viscosity of mucin formed by epithelial cells as well as the mucins of vitreous humor and synovial fluid. The viscosity of starch paste also is reduced rapidly both by vitamin C and by mucinase.

The reported phosphatase activity of vitamin C; the isolation of amolytic phosphatase, an enzyme which destroys the viscosity of starch paste; the slow reduction of viscosity of synovial fluid mucin by phosphatase, and the presence of phosphorus in the polysaccharide of synovial fluid mucin suggest a phosphate linkage in the aggregates of the high molecular weight polysaccharides which yield viscous solutions.

The above observations suggest too that vitamin C may be of significance in the metabolism of tissues containing mucins.

Blood volume and extra cellular fluid in infants and children. M. ROBINOW (by invitation) and W. F. HAMILTON. Department of Pediatrics and the Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta.

Blood volumes were obtained by the colorimetric determination of Brilliant Vital Red using standards made up in heparinized whole blood. Blood volumes were estimated and sufficient dye injected to make a dilution close to that of the standard. Dye was mixed with blood for the standard and injected at about the same time. After 3 to 4 minutes a sample of blood was withdrawn and centrifugalized with the standard. The plasma from the standard and from the sample were treated with 10 to 20 volumes of alcohol. This precipitated plasma proteins and adventitious hemoglobin and cleared up cloudiness due to lipemia. The centrifugalization left clear supernatant fluids which were almost always a perfect colorimetric match from which the dilution of the dye and hence the blood volume could be calculated. This method of determining Brilliant Vital Red in blood was described in this Journal (102: 551, 1932).

Blood volumes were determined on new-born infants. Plasma and cell volumes varied with the hematocrit which itself was very variable but the total blood volume was rather constant in relation to body weight.

After the simultaneous injection of sulfocyanate and Brilliant Vital Red into dogs, arterial blood samples were drawn every five seconds. The fluid available for dilution of the two substances was nearly the same during the pulmonary circulation, but as the sulfocyanate first returned from the systemic paths it was diluted by a volume becoming progressively larger for about 30 minutes. The dye concentration curve, on the other hand, suggested that mixing was nearly complete after two total circulations and that subsequent fall of dye concentration was due to other factors.

Blood volumes in a series of infants and children were determined simultaneously with "extra cellular fluid volumes" (volume of fluid available for dilution of NaSCN). Data obtained from normal children were related to body weight, height, surface and ideal weight. These data were compared with similar findings in various pathological conditions.

Further studies on the chondroitin growth factor. H. E. ROBINSON (by invitation), R. E. GRAY (by invitation) and L. A. CRANDALL, JR. Research Laboratories, Swift and Company, Chicago, and Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Our original observations (Robinson, Gray, Chesley and Crandall. *J. Nutrition* (In press)) that chondroitin sulfuric acid exerts a growth effect in chicks have been confirmed by several further experiments. The basal diet used in the earlier work has been improved to the extent that, with proper chondroitin addition, almost normal growth results. Since glucuronic acid is a constituent of the chondroitin molecule, a glucuronic acid concentrate was prepared from borneol glucuronate and calcium aldobionate was prepared from gum arabic. These materials exhibited only partial activity as compared to their chondroitin equivalence. This relative inactivity might be due to chemical structure variations between the compounds used and glucuronic acid in chondroitin.

Additional amounts of thiamin, nicotinic acid, isoscorbic acid, cholic acid, arginine, glucosamine, and high quality protein have been found inactive in the chondroitin-deficient basal diet.

The announcement of the discovery of "factor U" (Stokstad and Manning. *J. Biol. Chem.* 125: 687, 1938) excited our interest in the possibility that it might be identical with the chondroitin factor. However, the

addition of bran, a potent source of "factor U", to our basal ration was without effect. Chondroitin sulfuric acid did not prove effective in Stokstad's basal diet.

Chondroitin has been found to be a decided interfering factor in assaying animal source products for flavin content by a chick method. In general, it has been found that animal by-product protein concentrates are very rich sources of chondroitin or the basic factor which produces growth in our basal ration.

Chondroitin did not as directly interfere with the Sherman-Bourquin assay for flavin, but did exhibit an effect which appeared to be a flavin-sparing or substitutive activity.

The elimination of the effect of the chemical mediator of renal hypertension.

S. ROBBARD (introduced by L. N. Katz). Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

Previous work from this laboratory (1) has shown that the normal kidney ameliorates or causes a disappearance of hypertension due to unilateral renal ischemia. In this report, we present further evidence in support of this view. Trained dogs were used and the femoral blood pressure recorded with a Hamilton manometer.

Hypertension was produced by unilateral renal ischemia with Goldblatt's technique. In six dogs the ischemic kidney was then removed leaving the normal kidney in situ. The blood pressure changes were then followed during the immediate postoperative period. In all six animals the pressure returned to normal levels within six hours.

In seven other dogs with hypertension due to unilateral or bilateral renal ischemia, a total nephrectomy was performed. In this group the blood pressure did not return to normal until from 9 to more than 50 hours, averaging about 25 hours after the operation.

The difference in these two groups of animals cannot be explained on a neurogenic origin of the hypertension but can be accounted for by a humoral mechanism. In a control group of fourteen dogs with normal pressure unilateral and bilateral nephrectomy aside from a fleeting, neurogenic pressure elevation lasting about six hours had no effect on the blood pressure.

Our results also indicate that the mechanism of maintenance of the normotensive level is different from that of the hypertensive. The results confirm the view that hypertension of ischemic origin is dependent on the ratio of ischemic to normal kidney tissue and show further that the destruction, neutralization or elimination of the chemical mediator can be accomplished at a rapid rate only in the presence of kidney tissue. The evidence as to whether this is due to excretory or metabolic action on the part of the kidney is not complete.

(1) Proc. Soc. Exper. Biol. and Med. **37**: 722, 1938; Am. Heart J. (in press).

Influence of age on functional survival of severed mammalian nerves. W. M.

ROGERS and HORACE O. PARRACK (introduced by P. E. Smith). Departments of Anatomy and Physiology, College of Physicians and Surgeons, Columbia University, New York City.

In twelve adult cats of unknown age the sciatic nerve on one or both sides was cut at the level of the neck of the femur.

Electrical stimulation was used to determine the functional condition of the peripheral end. The threshold remained constant until the last few hours, then rose rapidly.

Maximal contraction decreased during the latter half of the survival period. This probably results from failure of individual nerve muscle units. Finally when very strong stimuli were applied to the nerve they evoked no muscular response, but the threshold for direct stimulation of muscle remained unchanged.

During these tests action potentials from degenerating and control nerves were compared. When stimulation of the cut nerve failed to cause a response of the muscle no nerve action potential was obtainable.

We believe that loss of irritability and conductivity in nerve fibers and not failure of the fatiguable substance in the motor end-plate is responsible for functional failure of the neuro-muscular mechanism. Terminal experiments on normal animals support this assumption. Both sciatic nerves were exposed before the animals were sacrificed. One nerve was removed to a moist chamber where action potentials were observed at five minute intervals. When no action potential was obtainable stimulation of the intact nerve on the opposite side failed to produce contraction in muscles it supplied.

Two spinal cats prepared by Dr. Root were used as controls for animals under anesthesia. Light anesthesia does not shorten the survival period of severed nerves.

Nerves were cut in rats ranging in age from five weeks to eighteen months. In the 5-week old animals, function disappeared after 23-24 hours in the 5 nerves studied. The survival time of the peripheral stump increased with age. In animals 7-9 months of age function lasted for 47-57 hours, 16 nerves being tested.

The adrenals in experimental hypertension. J. M. ROGOFF, E. NOLA NIXON (by invitation) and GEORGE N. STEWART (by invitation). Physiological Laboratory, University of Chicago, and Laboratory of Experimental Endocrinology, School of Medicine, University of Pittsburgh, Pa. (Read by title.)

Seven dogs were subjected to complete removal of both adrenal glands in addition to constriction of the main renal arteries by means of Goldblatt clamps. Some were treated by administration of salts (sodium chloride and citrate, occasionally also bicarbonate), alone or in combination with a commercial extract of adrenal cortex. In three experiments, no treatment whatever was employed. In all observations the blood pressure measurements were made by the carotid loop (Van Leersum) method. The cuff was adjusted by the same observer in every case and not less than 12 readings were made each time.

In 2 of these dogs, whose normal blood pressure was about 130 mm. and 135 mm. an adrenal was excised and the corresponding renal artery constricted at one operation; later the same was done on the opposite side. These dogs succumbed on the 4th day after the second operation, the blood pressure being as high as 234 mm. and 196 mm. (ave. 218 mm. and 186 mm.) respectively. The normal blood pressure in the third dog ranged from about 150 mm. to 160 mm. One adrenal was excised and the corresponding renal artery constricted. Later the renal artery on the opposite side was constricted. A marked hypertension was present when the second

adrenal was later excised; although the dog lived into the 9th day, there was no significant change in the level of blood pressure until 2 days prior to the death of the animal. The pressure reached 264 mm. on the 2nd day after the adrenalectomy and on the 8th day when in a semi-comatose condition and obviously moribund, the animal's blood pressure was still close to 200 mm. Hg.

Since hypertension which follows production of renal ischemia can be maintained in untreated animals that have been subjected to complete bilateral adrenalectomy, it seems obvious that neither the cortex or medulla of the adrenal plays an important role as an underlying cause of the hypertension. This is contrary to the results reported by certain other investigators.

An apparatus for constant intravascular injection of fluids at different rates.

J. M. ROGOFF. Laboratory of Experimental Endocrinology, School of Medicine, University of Pittsburgh, Pa. (Demonstration.)

A constant speed motor drives a nuggled disk by a hard rubber friction drive; the disk operates a pinion and gear reduction to a screw which is kept in constant motion, propelling forward the piston of one, two or three syringes. When syringes with a capacity of 50 cc. are used, adjustment can be made so that any quantity between 0.7 cc. and 7.5 cc. a minute can be delivered at a constant rate of flow against any pressure that might be met in the blood vessel. Further alteration of the range of quantities delivered can be accomplished by employing syringes of different capacity.

*Some effects of ether anesthesia on spinal dogs.*¹ WALTER S. ROOT and FERDINAND F. McALLISTER (by invitation). Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

The effect of one hour of ether anesthesia on: 1, heart rate; 2, blood pressure; 3, rectal temperature; 4, plasma proteins (refractometer); 5, hematocrit; 6, lactic acid (Friedmann, Cotonio and Shaffer), and 7, plasma volume (Gregersen et al., 1935; 1937; 1938; 1939), was studied on twelve spinal dogs, four to forty-six days after operation. The blood ether concentration was determined by Ruigh's method (personal communication).

In the control ether experiments, the animals showed the usual decrease in plasma volume with a corresponding increase in the plasma protein concentration (McAllister. *Am. J. Physiol.* **124**:391, 1938). The average blood lactate concentration rose 40 mgm. per cent. Following the ether excitement stage the systolic blood pressure (femoral artery; lumbar cord sectioned) remained at, or 10 to 20 mm. Hg. above, the pre-anesthetic level. The heart rate accelerated 100 to 150 beats per minute.

Up to ten days after transection of the spinal cord between CVII and TVII (the exact level in each case verified at autopsy) ether administration produced no change in either the plasma volume or the plasma protein concentration. The blood lactate and the hematocrit showed little or no increase. The systolic blood pressure fell 30-60 mm. Hg and the subsequent height of the blood pressure varied inversely as the blood ether concentration. Etherization of dogs with low cervical cord sections

¹ Aided by a grant (to M. I. Gregersen) from the Committee on Grants-in-Aid, National Research Council.

resulted in an increase in heart rate of 40-60 beats per minute. In animals with sections below TIV the heart rate increased to the same extent as in the control experiments.

It should be emphasized that the ether determinations give a reliable basis for comparing the depths of anesthesia in the normal and in the spinal animals. In both series of experiments the values ranged from 100 to 150 mgm. per cent of ether.

Histamine metabolism in the adrenalectomized rat. BRAM ROSE¹ and S. KARADY (introduced by J. S. L. Browne). McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

In previous work it has been shown that the ability of the intact rat to destroy or inactivate histamine is greatly diminished following adrenalectomy (Rose and Browne). It was noted that destruction of the histamine seemed to take place in tissues, such as the kidney, which do not contain histaminase in this species to a much greater extent than in those which do contain it, such as lung or intestine.

Further experiments have been carried out using the same method to show that the ability to inactivate histamine may be restored by the administration of Cortin and desoxycorticosterone acetate. Rats were maintained on normal saline and treatment was begun seven days after adrenalectomy. Using cortical extract (Wilson) of which 0.5 cc. daily is regarded as a maintenance dose, a dose of 2 cc. daily for three days followed by 2 cc. four times for one more day was found to restore this function, whereas 0.5 cc. daily for four days failed to do so. Using desoxycorticosterone acetate, it was found that 2.5 mgm. twice daily for three days followed by 5 mg. twice daily for one day was effective.

Since it has been shown that some mechanism other than histaminase must take part in the mechanism by which histamine is destroyed in vivo, observations were made on the effect of adrenalectomy on the histaminase content of lung tissue, from normal and adrenalectomized rats. The following method was used: Fresh tissue was ground up, weighed, and placed in 50 cc. Erlenmeyer flasks to which 20 cc. of phosphate buffer solution at 7.2 and 1000 γ of histamine (as base) were previously added. After mixing toluol was added and the whole incubated for 16 hours at 37°C. The flasks were then removed, the content made up to volume, and the histamine content assayed on the guinea-pig intestine preparation. In all experiments, controls were incubated at the same time. A diminution in the histaminase content of lung tissue from adrenalectomized animals was found, but this diminution is not sufficient in our opinion to account for the difference in the ability of the normal and adrenalectomized animal to inactivate histamine.

The production of hypertension by renal ischemia in the rat. BRAM ROSE¹ and PAUL WEIL¹ (introduced by J. S. L. Browne). McGill University Clinic, Royal Victoria Hospital, Montreal, Canada. (Read by title.)

In a series of hooded rats, renal ischemia was produced as described by Ryland by partial occlusion of both renal arteries performed by ligating the artery together with a wire of small diameter. After tying the ligature, the wire was removed.

Blood pressure determinations were made by a method which is essen-

¹ Aided by a grant from the Banting Research Foundation, Toronto.

tially that described by Byrom and Wilson in which the tail is placed in a plethysmograph, the arterial and venous blood vessels are quickly compressed by means of a small blood pressure cuff connected to a mercury manometer. The pressure is released slowly, and the point at which an increase in the volume of the tail is first observed is taken as the systolic pressure. The animals are all anaesthetized with ether.

In all 125 rats were studied; normal readings were taken for two weeks in a number of these as controls. As obtained by this method, the normal blood pressure is 80 to 100 mm. Hg, with occasional readings of 60 and 110.

Following the ligation, the response depended upon the degree of ischemia. In general a rise in the blood pressure was noted within 96 hours. Two types of response were noted, one in which there was a rapid rise within 36 hours to a pressure of 160–170 mm. Hg lasting six days, followed by a precipitous drop in pressure and death. In the second a more gradual rise ensued. In some of these animals the pressure remained at about 180. mm. Hg for several weeks.

Where no rise was observed, exploration of the animal usually revealed one necrotic kidney and one normal kidney or one ischemic kidney with the opposite one hypertrophied. Religation or removal of a normal or hypertrophied kidney with one ischemic kidney remaining was followed by a rise in blood pressure. In several cases where a transient rise was observed followed by a return to normal, laparotomy revealed an ischemic kidney with a hypertrophied kidney on the opposite side.

Histological studies have been made on tissues removed from hypertensive animals, and the effects of various sterols on the blood pressure of hypertensive animals are being investigated.

The 5th stage of neuromuscular transmission. A. ROSENBLUETH and J. V. LUCCO (by invitation). Department of Physiology, Harvard Medical School, Boston, Mass.

Stimulation of a motor nerve with a sufficiently high frequency leads to the following sequence of changes of tension: the initial rise (1st stage of transmission) is promptly followed by a fall (2nd stage), in turn succeeded by a further rise (3rd stage). A slow decline follows, which is the classical period of "fatigue" (4th stage). The tension will then remain very low for minutes or hours, but if the stimulation is continued a late rise will occur (5th stage). The tension may then attain high values and be sustained for hours.

The time course of the 4th and 5th stages is uninfluenced by dial anesthesia, by previous sympathectomy and by the load applied to the muscles. Both the time course and the tension developed are influenced by the frequency of stimulation and are different for gastrocnemius-plantaris and soleus.

The variations of tension are not correlated with corresponding changes in the motor nerve impulses. The changes in the muscle action-potentials are roughly parallel with the tension. The irregularities in the mechanograms during the 4th and 5th stages are due to alternation of the muscle fibers. The 5th stage subsides progressively during rest. The muscle does not revert to the 4th stage during this subsidence.

The data lead to the inferences that the 4th and 5th stages are synaptic phenomena and that they are due to different and independent factors.

Histamine, the chemical mediator for pain (?). SOL ROY ROSENTHAL and DAVID MINARD (introduced by G. E. Wakerlin). Tice Laboratories of the Municipal Tuberculosis Sanitarium and the Departments of Pathology, Bacteriology, Public Health, and Physiology, University of Illinois, Chicago.

Evidence is presented to show that histamine is liberated when the upper layers of the skin are stimulated in the threshold range when no gross or microscopic evidence of tissue damage is demonstrable. A histamine-like substance is recoverable from the anterior chamber of the rabbit's eye upon electrical stimulation of the cornea. This substance is liberated in direct proportion to the intensity of the stimulus. Histamine when injected intradermally or applied to the denuded skin (less epidermis and some cutis) or cornea causes pain. That the substance liberated is most likely histamine was shown by its action upon the intestinal strip of the guinea pig which action was not effaced by adding atropine to the bath, by its heat stability, its neutralization by histaminase, its dialysability through colloidal membranes and by the fact that thymoxyethyl-diethylamine which appears to be specific for histamine, neutralizes the action of the diffusates of stimulated skin and when injected subcutaneously abolishes universally the pain responses to pinching, pricking, cutting and lowers the electrical threshold of the skin markedly without affecting the somatic sensory nerve trunks. Histamine appears to be the chemical mediator for cutaneous pain.

The relative rôles of the extremities in the dissipation of heat under various atmospheric temperatures and relative humidities. GRACE M. ROTH (by invitation), BAYARD T. HORTON (by invitation) and CHARLES SHEARD. Section on Clinical Physiology, Divisions of Medicine and Biophysical Research, The Mayo Foundation and The Mayo Clinic, Rochester, Minn.

In previous investigations on human subjects, studies have been made on the vasomotor control of the loss of heat from the body under various changes in the environmental temperature while the relative humidity, in general, has been maintained at 25 per cent or 40 per cent. In the present study, data were obtained on four normal subjects in psychrometric rooms to determine the effects produced by changes in the relative humidity, in a comfortable range of environmental temperatures on the peripheral supply of the blood to the extremities as evidenced by changes in skin temperature. These investigations showed that relative humidity had little effect on the skin temperatures of the body, as indicated most sensitively by changes in the temperatures of the fingers and toes, when the body is subjected to fixed environmental temperatures ranging from 22°C. to 28°C., (72° to 82.5°F.) with a fairly wide range (35 to 70 per cent) of relative humidity. The dissipation of heat from the body, under the range of temperatures specified, is dependent chiefly on the atmospheric temperature and is little influenced by relative humidity.

A microphonic drop-counter. LEOPOLD ROVNER (introduced by H. Necheles). Department of Gastro-Intestinal Research, Michael Reese Hospital, and the Department of Physiology, University of Chicago, Ill. (Demonstration.)

We have devised a simple drop counter which permits exact individual counting and recording of drops, even at high speeds, by means of a direct and easy set-up that obviates accurate mechanical or optical alignment in the laboratory procedure, and which does not affect the drops of secretion by electrolysis.

The instrument is a microphonic counter consisting of a microphone insensitive to room sounds, and an amplifier-relay-counter circuit which records the single mechanical pulses delivered to the microphone by the falling particle.

The particle or droplet falls from a height of two to three inches upon a light weight disk (about one inch diameter) which is directly connected by a light, rigid arm to the element of a sound pick-up microphone. (We have used a carbon button and now use a magnetic microphone pick-up.)

Electrical pulses from the microphone circuit are amplified by a 25A7G tube (A.C. or D.C.) and sent from the plate circuit of the tube through a matching transformer into a magnetic relay. Opening of the switch points on the relay sends an energizing current through a solenoid counter which records the drop by number on a counting wheel. A plug-in switch permits one, while recording the number of counts, to energize a magnetic recorder on a smoked drum for obtaining a record, say, of drops of secretion coincident with other physiological data being recorded on the drum.

The instrument is positive in its action and responds only to mechanical agitation of the microphone extension. The arm bearing the sensitive disk extends out and downward at an angle from the microphone element and can be lowered into a glass beaker used for quantitative collection of all of the falling material.

The apparatus can be built from radio apparatus parts at a nominal cost.

Brain potential changes during cyclopropane anesthesia. MORTON A. RUBIN and HARRY FREEMAN (introduced by R. G. Hoskins). The Memorial Foundation for Neuro-Endocrine Research and the Research Service of the Worcester State Hospital, Worcester, Mass.

A characteristic sequence of potential patterns is observed during cyclopropane anesthesia which differs somewhat from that seen in normal sleep. In sequence, the patterns are: 1, alpha (awake); 2, slowing of alpha waves and increase in amplitude; 3, slowed alpha waves plus irregular delta waves; 4, increased irregularity and further slowing of frequency; 5, regular, high voltage 2-4/sec. waves; after cyclopropane is stopped; 6, stage 5 persists for a very short time and then reverts to stage 4; 7, marked decrease in voltage and occasional 14-18/sec. waves; 8, 14/sec. waves of increased voltage and per cent time occurrence; 9, 14/sec. waves plus alpha waves of normal and slowed frequency; 10, alpha (awake).

Brief fluctuations in brain potential pattern may occur superimposed on an unidirectional trend during anesthesia and on recovery from it. The pattern sequence on recovery does not mirror the sequence during anesthesia.

The potential patterns of a given cortical region during anesthesia are similar in both hemispheres. The patterns of the various areas within a hemisphere differ consistently in several respects.

It appears that the cortical neurones responsible for the alpha rhythm are capable of beating at many frequencies. In addition, the data support the view that the potential changes of the various cortical regions are comparatively independent.

Further observations on the effect of ligation of the pancreatic ducts upon the blood and liver lipids of the dog. SAUL H. RUBIN (introduced by Elaine P. Ralli). Laboratories of the Department of Medicine, New York University College of Medicine, New York City.

In confirmation of a previous report from this laboratory, we find that ligation of the pancreatic ducts leads to the appearance of fatty livers in dogs. As the fatty infiltration into the liver advances, the postabsorptive plasma lipids fall, the most marked decrease occurring in esterified cholesterol. These observations argue against the theory that a fat-metabolizing hormone is elaborated by the pancreas.

On the other hand, we have confirmed the observation of Dragstedt that the minimal effective prophylactic dose of pancreas for the depancreatized, insulinized dog is about 25 grams, containing about 60 mgm. of choline, while the minimal dose for choline is 2 grams daily. The administration of 25 grams of pancreas daily causes a gradual increase in plasma lipids, notably in esterified cholesterol.

The production of xanthomata in rabbits. H. P. RUSCH, C. A. BAUMANN and B. E. KLINE (introduced by W. J. Meek). Physiology Laboratory, University of Wisconsin Medical School, Madison.

Eight young adult female albino rabbits were given a stock diet containing 0.23 per cent cholesterol. Six similar rabbits given stock ration only, served as controls. Xanthomata developed in the subcutaneous tissue of the feet of seven of the eight rabbits receiving cholesterol, while none of the controls were affected. While one animal developed a xanthoma after 6 months, most of the nodules were noted after 10 months of cholesterol feeding. These continued to increase in size until the death of the animals. The nodules were usually found on the feet but an occasional one was also observed about the knee and elbow joint. The pads of all feet were invariably involved, becoming swollen, tender and denuded. The xanthomata ranged in size from that of a pea to 8 cm. in diameter, the average size being 1-3 cm. The tumors were soft, rounded and either white or yellowish in color. They consisted of soft fatty tissue entangled in varying amounts of tough connective tissue. Histological examination revealed masses of large foamy cells loaded with lipoidal material. Chemical analysis of 12 nodules indicated that fat constituted 46.4 per cent and total cholesterol averaged 4.57 per cent of the dry weight. The blood cholesterol averaged 6.17 mgm./cc. while in the control animals, the average was 1.42 mgm./cc. Extraneous factors such as ultraviolet irradiation and the intravenous injection of papilloma virus in a few of the rabbits in no way affected xanthoma formation. It appears that a hypercholesterolemia of long duration is in itself responsible for the production of xanthomata in rabbits.

Iodoacetic acid and anaerobic muscular contraction. JACOB SACKS. Department of Pharmacology, University of Michigan, Ann Arbor.

The first reactions taking place in contraction of isolated muscles of iodoacetate poisoned frogs are the conversion of phosphocreatine to hexose-monophosphate and some phosphocreatine hydrolysis. Contracture begins when practically all the phosphocreatine has broken down, and adenosine triphosphate breakdown begins at this point.

In complete rigor, hexosediphosphate is formed by conversion of the

monophosphate into diphosphate. Inorganic phosphate is utilized as readily as adenosine triphosphate for this reaction.

No evidence was found for the presence of triosephosphate, although it is stated by Meyerhof and Lohmann that iodoacetic acid does not affect the formation of this substance from hexosediphosphate in extracts.

The data indicate that the formation of hexosemonophosphate is a process independent of the formation of lactic acid, rather than an intermediate step in the formation of the latter substance.

Hexosemonophosphate formation directly from phosphocreatine and diphosphate formation from inorganic P have not been found in muscle extracts. From all these findings it is concluded that caution is necessary in transferring findings in muscle extract to contraction in the intact muscle.

The influence of lung distention on gastric hunger motility in the bullfrog.

R. E. SCANTLEBURY (by invitation) and T. L. PATTERSON. Department of Physiology, Wayne University College of Medicine, Detroit, Mich.

Incident to our series of studies on the alterations in gastric motility following distention of hollow visceral organs the bullfrog was selected for lung-stomach observations because of its tubular lung. Chronic experiments were performed. A stomostomy (Patterson. *Am. J. Physiol.* **40**: 140, 1916; *ibid.* **42**: 61, 1916) was made in the bullfrogs and the normal motility of the empty stomach was recorded by the balloon method using a water manometer. A dual operation was then performed consisting first of a decerebration followed by an artificial lung fistula (pneumostomy) made in about the mid-flank and one to one and one-half centimeters to the right of the dorsal mid-line. The tip of the lung was carefully drawn through the incision and a small slit made to admit a condom balloon of suitable size to fit the lung cavity. This balloon was then introduced and the tip of the lung was tied to the end of the cannula to which the balloon was also attached and the incision closed with two rows of sutures. The presence of the uninflated balloon in the lung did not produce inhibition for in three to four hours normal gastric motility was usually resumed. A fluid pressure-regulating system was employed for developing the desired distention-pressure in the lung which was recorded simultaneously with gastric motility. Sufficient and sudden lung distention will completely inhibit the gastric contractions, the onset of which is immediate but on the release of the lung pressure recovery is, in most cases, rapid and complete. Lower pressures will diminish the amplitude of the contractions and show a lengthening of certain of the intervals of rest between the individual contractions. Still lower pressures introduced into the lung-balloon are ineffective. The lung shows within certain limits a degree of adaptability when the lung pressure is increased very, very gradually.

The non-protein nitrogen content of the plasma during adaptation to various agents. VICTOR SCHENKER (introduced by Hans Selye). Department of Anatomy, Histology and Embryology, McGill University, Montreal, Canada.

Previous experiments have shown that if an animal is forced to adapt itself to a damaging agent, characteristic morphological, functional and biochemical changes occur during the first 24-48 hours, that is, during the period of the "alarm reaction". Later however, when the animal enters

into the "stage of resistance", these changes disappear or are even reversed. Thus for instance, the blood chlorides, blood volume and blood sugar which are decreased during the alarm reaction are considerably above normal during the stage of resistance (Selye, H. *Arch. Internat. de Pharm. et de Thérap.* 60: 259, 1938). The purpose of the present investigation was to establish the blood N.P.N. changes during adaptation.

Twenty-four adult albino rats were used for this purpose. Six of them were exposed to cold (+3 to +5°C.), six were treated with three daily injections of 0.3 cc. of a 4 per cent formaldehyde solution and a third group of six was forced to exercise for one hour, three times daily in drum cages having a diameter of 12 inches and revolving at the rate of 18-22 revolutions per minute. The remaining six animals were left as untreated controls. Blood was taken from the tail vein before beginning the experiment and 1, 6, 12, 24 and 59 hours after initiation of the treatment. It was observed that during this period, while the symptoms of the alarm reaction developed, the plasma N.P.N. (Micro-Folin-Wu modified for Evelyn's photo-electric colorimeter) rose considerably in all groups irrespective of the type of treatment given. In many cases, the rise amounted to 100-200 per cent of the initial value. Ten days after initiation of the treatment when the animals became resistant to these agents, the N.P.N. values returned to normal. In the untreated control group, the variations in the N.P.N. content of the serum throughout the experiment were only insignificant.

It is concluded that in these experiments, the rise in the N.P.N. content of the plasma is a characteristic feature of the alarm reaction and not the result of a specific pharmacological action of the agents used, because this change appears irrespective of the nature of the agent and disappears in spite of continued treatment as soon as adaptation occurs. Contrary to many other changes occurring during adaptation, the initial rise in N.P.N. is not reversed after resistance is acquired but simply returns to normal.

A comparison of the secretory response of jejunal and ileal segments. M. J. SCHIFFRIN (by invitation) and E. S. NASSET. Department of Vital Economics, University of Rochester, Rochester, N. Y.

Juice was collected from normally innervated segments of jejunum and ileum. The following determinations were made hourly: 1, volume; 2, pH; 3, amylase; 4, sucrase; 5, erepsin; 6, lipase; 7, total CO₂, and 8, chloride (in some experiments). The dogs were kept on a constant diet and were always given a standard meal whenever the effect of feeding was to be observed.

In the jejunum the enzyme production on fasting days declined throughout the day as did the volume of juice. For 3 or 4 hours after a meal the enzyme production declined, then began to rise and at the sixth or seventh hour exceeded the initial values; the volume of juice declined steadily. The secretion was always alkaline with 84 per cent of the pH values between 7.6 and 8.4. The extremes were 7.0 and 8.6. In confirmation of earlier work the chloride concentration was found to remain rather constant with 85 per cent of the values lying between 130 and 150 millimols per liter. The average total CO₂ concentration was 9.9 millimols per liter for fasting days, being increased to 14.8 after feeding.

The enzyme production of the ileum was a third to a reduction in concentration of the enzymes and a diminished amount of secretion. Qualita-

tively the response to food was similar to that of the jejunum but at no time during the day did the enzyme production equal the initial value. On fasting days there was not the regular decrease in enzyme production seen in the jejunum. The ileal secretion was always more alkaline than the jejunal secretion by about one pH unit. In marked contrast to the jejunal secretion the total CO_2 content of the ileal juice was 60 to 67 millimols per liter and was not much affected by feeding.

Electrolyte content of the axoplasm of squid giant nerve fibers. FRANCIS O. SCHMITT and RICHARD S. BEAR (by invitation). Washington University, St. Louis, Mo.

Axoplasm virtually uncontaminated with other tissue components was obtained from squid giant nerve fibers (diameter ca 500μ) by extrusion from the cut ends of teased and ligated single nerve fibers. Development of rapid methods for preparing fibers, for separating axoplasm from sheath, for weighing each component, and for quantitative estimation of each constituent permitted *direct* determination of axon electrolytes and other substances.

In milliequivalents per gram of fresh axoplasm (Ax) or per ml of squid blood (Bl), the results for electrolytes were: total base, Ax = 0.56, Bl = 0.60; potassium, Ax = 0.28, Bl = 0.015; chloride, Ax = 0.12, Bl = 0.47. These figures confirm the conclusions of Fenn et al. drawn from experiments on intact frog and lobster nerves regarding the relatively high concentration of potassium and the relatively greater deficiency of inorganic anions in the interior as compared with the exterior of nerve fibers. The deficiency of inorganic anions in axoplasm is particularly striking, chloride ions being only about one fourth as concentrated as inorganic cations. From estimates of total solid, total phosphorus, and protein and non-protein nitrogen of squid axoplasm it is improbable that this large anion deficiency is made up by ionized protein groups or by phosphates or other simple inorganic anions.

Extracts of lobster nerves made by cutting, freezing and thawing the nerves in distilled water show a similar anion deficiency which is little changed by removing the protein by heat coagulation. Heat and trichloroacetic acid precipitate the same relative amounts of nitrogen from such extracts, leaving about two thirds of the total nitrogen in solution. Titrations of such protein-free extracts with the glass electrode show acid-base binding which is qualitatively similar to that of complex organic acids, such as the nucleic acids. This fact, along with the unusually high NPN of nerve axoplasm and extracts suggests that the anion deficiency is made up by components which contribute to this high NPN.

An optical method for the determination of the thickness of the limiting envelope of mammalian erythrocytes. FRANCIS O. SCHMITT and DAVID F. WAUGH (by invitation). Washington University, St. Louis, Mo. (Demonstration.)

Mammalian erythrocytes may be deposited on a glass slide, the hemoglobin removed by lysis, and the remaining stromata dried. If the refractive index of the glass is high enough (1.70-1.90) the dried stromata show interference colors which can be compared with the interference colors produced by thin films of barium stearate (built up on glass of the same refractive index) according to the method of Blodgett and Langmuir.

Since we find that the refractive index of the stromata is very close to that of stearate films, and since, like stearate films, the membranes are uniaxial with optic axes perpendicular to their surfaces (Schmitt, Bear and Ponder, 1936), this comparison is justified.

The stearate step film (built up in double layer steps) and the dried stromata are examined with separate microscopes, each fitted with a vertical illuminator. A single intense, filtered light source illuminates both microscopes. By means of a comparison ocular, the color and intensity of the light reflected from the surfaces of the cellular membranes and of the film steps may be compared. It is thus relatively easy to determine which step of the stearate film most closely matches the particular double membrane under observation. Knowing the thickness of the film step, that of the membrane may be estimated with an accuracy of ± 12 AU. Since many cells are measured in each preparation, the average thickness is correspondingly more accurate. Requisite for success with this technique is the use of matched vertical illuminators, objectives and oculars, and a critical matching of the reflectivities of the surfaces of the two slides being compared.

Since the dried membranes are not strictly uniform in area under varying conditions, the diameters of the cells used for thickness determinations are also measured and the volumes of the dried double membranes calculated. Dividing these volumes by the average surface area of the cells as determined on fresh cells gives a value corresponding to the thickness of the membrane in its normal state of distension.

Oxygen therapy in post-puncture and barbiturate reactions. J. G. SCHNE-DORF (introduced by C. A. Dragstedt). Departments of Surgery and Pathology, Henry Ford Hospital, Detroit, Mich.

Reactions following barbiturate anesthesia and the aseptic cisternal withdrawal and replacement of cerebrospinal fluid were studied upon normal dogs under pentobarbital and amytal anesthesia.

It was observed that pentobarbital and amytal anesthesia (30-35 mg. per kilogram body weight intravenously) produced a decrease in the oxygen saturation of the arterial blood which in some cases persisted for more than four hours. Over an eight hour period there occurred only a slight elevation of cerebrospinal-fluid pressure (23-29 mm.) above normal and no significant alteration in spinal fluid protein or cells.

The slow aseptic cisternal withdrawal and replacement of 8 cc. of cerebrospinal fluid caused an increase in the cerebrospinal fluid protein (122 mg. per cent) and cells (1,546) and a marked elevation of cerebrospinal fluid pressure (124-156 mm.), body temperature (3.9-4.2°F) and respiration (9-19) above normal.

Nasal oxygen therapy restored the arterial oxygen saturation. The amount of protein was reduced (53 mg. per cent) and the number of cells in the cerebrospinal fluid was also reduced (672). Elevations in cerebrospinal fluid pressure were moderate (69-93 mm.) and returned to lower levels sooner than in the dogs which did not receive oxygen. Elevations of temperature and respiration did not occur.

Adaptation to climate and cardiac outputs while standing. J. C. SCOTT and H. C. BAZETT. Departments of Physiology of the Hahnemann Medical School and University of Pennsylvania, Philadelphia. (Read by title.)

Observations on the effect of climatic changes on blood volume and cardiovascular reactions have already been reported. We have also previously reported that cardiac outputs while standing vary at different seasons of the year, but cannot be correlated with the temperatures at the time of observation. In an experiment of two weeks duration in an air-conditioned room in June 1938 standing cardiac outputs were measured by acetylene on 42 occasions on two subjects. The subjects were already partially adapted to warm weather before the start of the experiment. Of these observations 9 were made under basal conditions in a room maintained warm (about 32.4°) and 11 later under similar conditions in a room maintained cool (about 21.1°). The average cardiac outputs per square meter per minute for the warm conditions was 1.95 liters with a standard deviation of ± 0.24 ; the average for the cool room was 1.63 ± 0.13 . An additional 10 observations were made in the warm room under non-basal conditions in the afternoon and a similar series of 8 in the cool room. The mean value of the series in the warm room was 1.95 ± 0.15 , in the cool room 1.79 ± 0.17 . The four values obtained in the cool room within 24 hours of the change in temperature have been excluded from the averages; these values tended to be high. The differences between the averages for the two conditions have been analyzed according to Fisher's t-values. The probability of the difference being due to mere chance is less than 1 in 100 under basal conditions and less than 1 in 20 under non-basal conditions. Increases in blood volume in response to continued warmth are considered to assist in the maintenance of cardiac output while the subject stands. The acetylene samples were taken after 12 and 18 seconds of rebreathing. Whatever the errors from recirculation, they should have been approximately constant. "Indices" of 1.6 and 2.0 are obtainable in the same subjects with the same procedure provided that climate conditions differ.

The effect of vagotomy on the external secretion of the pancreas. V. BROWN SCOTT, DONALD H. MCCARTNEY and JOHN S. GRAHAM (introduced by P. M. Harmon). Department of Physiology, Indiana University School of Medicine, Bloomington.

Considerable evidence has accumulated for the existence of vagus motor nerves to the pancreatic ducts. Since fluctuations in the drop-by-drop secretion of pancreatic juice were known to occur it seemed possible that such might result from contraction and relaxation of the pancreatic ducts effected through these vagus nerves.

In order to investigate this possibility the secretion of pancreatic juice was studied in 6 chronic fistula dogs (fistula of the major pancreatic duct after the method of Dragstedt). These animals were trained to lie quietly on a table and after a 24 hr. fast the drop-by-drop secretion was recorded by an automatic drop counter for a period of 2 hrs. Records obtained before and after intrathoracic vagotomy showed no differences in the magnitude or rate of fluctuations in this secretion. From these results it appeared evident, therefore, that these fluctuations in the fasting pancreatic secretion could not be explained upon the above hypothesis.

Comparative studies on the external secretion of the pancreas in fasting chronic fistula dogs. V. BROWN SCOTT, JOHN S. GRAHAM and DONALD H. MCCARTNEY (introduced by P. M. Harmon). Department of

Physiology, Indiana University School of Medicine, Bloomington.
(Read by title.)

The fasting external secretion of the pancreas has been studied in 22 chronic fistula dogs of the following types: 1, fistula of a duodenal pouch receiving the major pancreatic duct (Dragstedt); 2, fistula of the major pancreatic duct transplanted to the skin of the abdomen (Inlow); 3, fistula of the major pancreatic duct by cannulation (Elman and McCaughan). The accessory ducts were not disturbed in any of these fistulae. Stainless steel pouch cannulae satisfactorily replaced the gold plated ones used by Dragstedt.

All observations were made upon dogs which were trained to lie quietly upon a table; the animals had fasted 24 hours. The drop-by-drop fasting pancreatic secretion was recorded by an automatic drop counter. Under these conditions 10 Dragstedt fistula dogs secreted an average of 1.49 cc. per kgm. per hr., 6 Inlow fistulae 1.23 cc. per kgm. per hr., while 6 of the Elman and McCaughan type averaged only 0.52 cc. per kgm. per hr. The latter type fistula did not prove satisfactory for extended observations because of the frequency with which the cannulae pulled out of the pancreatic ducts. Analysis of the drop-by-drop secretion demonstrated fluctuations in the rate and magnitude of such secretion. These fluctuations were of the same general type in all the fistulae studied; the chief differences were in the magnitude of these fluctuations which was much less in the Elman and McCaughan type. In our experience the external secretion of the pancreas seemed to be reduced by cannulation of the major pancreatic duct.

Characteristics of O₂ transport in the blood of the mink (Mustela vison).

WALTER J. SCOTT (by invitation), LAURENCE IRVING and V. SAFFORD (by invitation). Edward Martin Biological Laboratory, Swarthmore, Pa.

The average oxygen capacities of the blood of normal mink was 26.0 ml per 100 ml. In the cells the average oxygen capacity was 46.6 ml per 100 ml. This oxygen capacity of the blood is considerably larger than in the blood of man, the dog and common laboratory animals, but not so large as in the seal. The concentration of hemoglobin in the erythrocytes of the mink is only slightly greater than usual for common laboratory mammals.

Oxygen dissociation curves conformed with the common picture for mammals. At 50 per cent saturation and $P_{CO_2} = 40$ mm. the PO_2 was 37 mm., which is somewhat larger than for the blood of man and the dog. The effect of CO_2 upon oxygenation (Bohr Effect) was similar to the common mammalian condition. Between P_{CO_2} 30 and 60 mm. the change in PO_2 for half saturation was 9 mm. or $\frac{\Delta PO_2}{\Delta P_{CO_2}} = \frac{9}{30}$.

In regard to O_2 capacity the blood of mink exceeds common land animals and approaches the large capacity of a few diving animals. Mink are good divers, but for their size they are also exceptionally strong and active on land. Their large oxygen capacity may be associated with either diving or generally intense activity.

Growth and development of albino rats under treatment with thymocrescin.

ALBERT SEGALOFF (by invitation) and WARREN O. NELSON. Department of Anatomy, Wayne University, College of Medicine, Detroit, Mich.

Asher has reported that in rats on deficient diets a highly purified thymus extract designated "Thymocrescin" will produce marked acceleration of growth, both of the soma and of the gonads. More recently Rowntree and his co-workers have reported marked acceleration of growth and differentiation in rats treated over successive generations with thymus extract (Hanson).

This work was undertaken in an attempt to bring together the work of these two groups by administration of thymocrescin to successive generations of albino rats.

Eight adult albino rats were divided into two groups of two females and two males each. The animals of one group were injected, subcutaneously, with 5 mg. per day of thymocrescin prepared after the method of Asher, the other group of animals serving as controls. In three further generations bred from each of these two groups no significant differences were noted in growth, opening of the ears, eruption of teeth, opening of the eyes, descent of the testes or vaginal canalization. Neither were there significant differences in organ weight at 70 days.

Each generation beyond the original animals contained 50 to 60 individuals reared through the observation period of 70 days. All animals were maintained on the laboratory stock diet of Bal-o-Ration and water ad libitum, with the addition of lettuce daily.

Attempts to reproduce the growth or gonodotropic effect reported for the extract showed no effect in animals on a synthetic B (thiamin) deficient diet, or on a horse meat diet suggested by Asher. No effect was found even on dosages of 10 mg. per day.

This work is being continued through further successive generations beyond the four generations presented above.

Effect of adaptation to various damaging agents on the ovary and the estrous cycle of the rat. HANS SELYE. Department of Anatomy, Histology and Embryology, McGill University, Montreal, Canada.

Experiments on adult female "hooded" rats show that during adaptation to various damaging agents such as muscular exercise, cold or toxic doses of various drugs, the estrous cycles of the rat are interrupted and the ovary decreases in size. The adrenals of these animals, on the other hand, showed an excessively developed cortex. It seems probable that this is due to a "shift" in the hormone production of the pituitary such as has previously been described (H. Selye and J. B. Collip, *Endocrinology* 20: 667, 1936) to occur in malnutrition. It appears that in a case of emergency, the pituitary produces more adrenotropic hormone in order to increase the adrenal cortical hormone production which is important for adaptation but at the same time, the hypophysis produces less gonadotrophic hormone.

Detailed histological studies of the ovaries of these animals reveal that even after an uninterrupted diestrous period of 3-4 weeks, fresh corpora lutea may be present in the ovary. Since corpora lutea in various stages of development were found in these ovaries simultaneously, it seems possible that the absence of vaginal estrus is due to the fact that only a few follicles matured at any one time, so that the estrin concentration in the blood was never sufficient to induce vaginal cornification in the presence of active corpus luteum tissue. This disturbance of the cycle could be cured in many cases by unilateral ovariectomy. Following this

operation, fairly regular vaginal cycles reappeared in spite of continued treatment with the above mentioned damaging agents. Possibly the gonadotrophic hormone production sufficed under these circumstances to stimulate one ovary in a normal manner, although it did not suffice to stimulate them both. Another conceivable explanation would be that partial castration increased the gonadotropic hormone production of the hypophysis even in these damaged animals.

These experiments indicate that corpus luteum formation can occur without being preceded by external signs of estrus. These cases probably represent the counterpart to the so-called anovular estrus periods and might be referred to as anestrus ovulations.

On the renal tubular excretion of creatinine in normal man. JAMES A. SHANNON and HILMERT A. RANGES (by invitation). Department of Physiology, New York University College of Medicine and Third (New York University) Division of Bellevue Hospital, New York City.

When the plasma creatinine is raised by the administration of this substance to 5-15 mgm. per cent, the "creatinine"/inulin clearance ratio averages 1.4, which ratio is believed to characterize accurately the excretion of true creatinine. The fact that further elevation of plasma level (50-150 mgm. per cent) reduces the clearance ratio towards 1.0 is explicable on the basis of a limited capacity of the tubules to excrete exogenous creatinine. Our present observations lead us to believe that after the administration of creatinine some of this substance is changed in the body to an unidentified chromogenic substance the clearance of which is lower than that of true creatinine, and approximately that of inulin. This conclusion is based on the following facts: 1. The creatinine/inulin clearance ratio does not fall when the plasma level of creatinine is maintained at 5-15 mgm. per cent by constant intravenous infusion (5 hours) 2. The clearance ratio does fall (from 1.4 to 1.1-1.2) after the administration of a single dose of creatinine. This decrease in ratio requires the lapse of a considerable interval of time. 3. After falling in this manner, the clearance ratio can be immediately restored to approximately its initial value by the intravenous administration of sufficient creatinine to raise the plasma level from 5-8 to 15-20 mgm. per cent. This last fact demonstrates that the decrease in ratio after single doses (2) is not due to fatigue of the tubular excretory mechanism. On the other hand, (1) argues against the interpretation that (2) is due to accommodation of the excretory mechanism after initial excitation.

Our experiments to date give no indication of what changes creatinine may undergo in the body. Our observations are in conflict with the belief that endogenous chromogenic substance is entirely creatinine, inasmuch as they support the view that the true creatinine clearance at low plasma levels is substantially higher than the inulin clearance. There is no evidence, however, indicating that the quasi-creatinine substance, which we believe to be formed from exogenous creatinine, and endogenous creatinine are identical, even though the clearances of both substances are close to the inulin clearance.

The effect of carbon dioxide upon the dynamic constants of muscle. HERBERT SHAPIRO. University College, London, and Clark University, Worcester, Mass.

The force-velocity relationships in muscle may be described quantitatively by an equation developed from heat measurements (Hill, Proc. Roy. Soc. B, **126**: 136, 1938), viz., $(P + a)(V + b) = b(P_0 + a)$, where P and V are respectively the relatively steady tension in grams exerted during shortening at V cm/sec, a the heat of shortening per cm. shortening, and b is a constant of proportionality representing the increase in rate of total extra energy liberation per gram decrease of load. As reported elsewhere (Shapiro, Anat. Rec. **72**: 66, supplement 1938) the relationship can be verified by a mechanical method, and the constants obtained are of magnitude similar to those yielded by the heat measurements. In the present series of experiments the influence of CO_2 in particular was investigated. Measurements were made of the force-velocity function in O_2 , and then in CO_2 , (generally 5 per cent CO_2 , 95 per cent O_2) of sartorii of the Hungarian bull frog, *R. esculenta*, the muscles being equilibrated in the different gases at constant temperature for an hour or longer before measurement. It was found that the equation applied to muscles in the presence of CO_2 , but with this chief difference, that the value of b was reduced, irrespective of the temperature of the experiment. The effect was rather incompletely reversible. Muscles exposed to 30 per cent CO_2 gave tensions too low to enable quantitative determinations. A possible interpretation of the significance of the alterations in the value of the constant b is, that b is related to the rate of chemical energy liberation for utilization by the contractile substance; here, CO_2 might so alter the sarcoplasm as to slow down the energy providing chemical processes, possibly by shifting the pH to a less favorable region for the enzymes concerned. A supplementary effect might arise from a direct action on the contractile mechanism.

The effect of anterior pituitary injections on the blood acetone bodies of adrenalectomized rats. REGINALD A. SHIPLEY (introduced by Joseph T. Wearn). Department of Medicine, Western Reserve University School of Medicine, and Lakeside Hospital, Cleveland, O.

Fasting adrenalectomized rats have been tested for their blood acetone body response after injections of crude anterior pituitary extract by the analyses of samples of tail blood taken before and after injection. Graduated doses of extract were given to groups of both adrenalectomized and normal rats in order that the respective assay curves could be compared. The adrenalectomized rats were sensitive to the extract but their response was only one half to one third that of unoperated rats when a comparison was made on the basis of the size of dose necessary to produce a given response.

Simultaneous blood and urine determinations were made in adrenalectomized rats receiving anterior pituitary extract. The results suggest that ketonuria fails to occur in these animals because the rise in blood acetone bodies is insufficient to exceed the urinary threshold.

Factors limiting visual resolution. SIMON SHLAER, EMIL L. SMITH (by invitation) and AURIN M. CHASE (by invitation). Laboratory of Biophysics, Columbia University, New York City.

Hartridge (J. Physiol. **57**: 52, 1922) advocated the view that intensity discrimination in the "deteriorated" image, resulting from chromatic aberration and diffraction, limits visual resolution. We have attempted

to evaluate experimentally the limiting factors in the case of two test objects: 1, the grating, and 2, Landolt's "C."

1. Shlaer (J. Gen. Physiol. **21**: 165, 1937) has shown that Hartridge's notion is invalid for the grating; that in white light the pupil must be large enough (2.3 mm. diameter) to transmit the first order spectrum resulting from the grating up to 630 m μ in order not to be the limiting factor; and that when the pupil diameter is not limiting the separation between retinal cones is the final factor. It follows then that with a pupil of 2.35 mm. the red end of the spectrum should give submaximal visual acuities. Using the Eastman Kodak "Monochromat" series of filters, we measured the maximum resolution in different portions of the spectrum. We find the maximum visual acuity to be progressively lowered in light of longer wavelengths beginning with the orange. At the extreme red end of the spectrum the drop is about 15 per cent. From this we have computed the size of pupil necessary to give maximal visual acuity to be 2.8 mm. Such a pupil transmits the first order spectrum up to 760 m μ . A 3 mm. pupil when tried did give maximal values.

2. If Hartridge's notion holds for the "C" test object, then improved resolution should obtain with monochromatic light, and with increased pupils. Measurements of maximum visual acuity in different portions of the spectrum and with 2 and 3 mm. pupils gave identical values. Measurements were then made with a test object $\frac{1}{2}$ as bright as the field. This is equivalent to a reduction of the intensity discrimination fraction by 50 per cent. Visual acuity under these conditions dropped only about 12 per cent. Measurements were then made in white, blue, and yellow light and with 2 and 3 mm. pupils. The combination of yellow monochromatic light and a 3 mm. pupil raised the visual acuity half way to normal.

Effect of oxygen tension of inspired air on the physiological response of normal subjects to carbon dioxide. NATHAN W. SHOCK and MAYO H. SOLEY (introduced by C. D. Leake). Divisions of Physiology and Medicine, University of California Medical School, Berkeley and San Francisco.

While in general the effects of breathing various concentrations of carbon dioxide have been studied rather extensively, there have been few reports of any alteration in response when the carbon dioxide is mixed with 90 or more per cent oxygen, rather than with 21 per cent oxygen.

Several writers have stated specifically that the concentration of oxygen makes no difference in the degree of hyperpnea caused by breathing carbon dioxide. In some experiments that we have been conducting to ascertain the respiratory effect of small concentrations of carbon dioxide, it was accidentally discovered that the use of oxygen, instead of air, with the carbon dioxide, augmented the hyperpnea considerably. We studied a group of normal subjects who had rested until their respiratory volumes had become stabilized, and then breathed a mixture of 1 per cent carbon dioxide and 21 per cent oxygen until their respiratory volumes again reached a level. This experiment was repeated using 1 per cent carbon dioxide and 99 per cent oxygen. In all the experiments the average increment in the respiratory volume was greater when the subjects breathed 1 per cent carbon dioxide in 99 per cent oxygen than it was when they breathed 1 per cent carbon dioxide in 21 per cent oxygen. Similar results were found when one-half per cent and when 2 per cent carbon

dioxide were used. In some of the subjects there was no statistically significant difference, due to the fact that their respiratory volumes were not stable even after a prolonged period of rest. In certain subjects this increased response to carbon dioxide mixed with oxygen is great enough to be of clinical importance, and should be considered when carbon dioxide is administered for therapeutic purposes.

*In vitro effect of insulin on respiration of minced mammalian muscle.*¹ E.

SHORR and S. B. BARKER. Department of Medicine, Cornell University Medical College and the New York Hospital, New York City.

Krebs et al. (Biochem. J. **32**: 913, 1938) have recently reported an in vitro effect of insulin (B.D.H. insulin hydrochloride) on the oxygen consumption of minced pigeon breast muscle in the presence of citrate and boiled muscle juice, using a phosphate buffer. Respiration fell less rapidly in this system than in control experiments with phosphate alone, or with added muscle juice and citrate in the absence of insulin. Differences of as much as 100 per cent were observed. The effect was proportional to the concentration of insulin used—0.05 to 5.0 mgm. per cent.

Because of the potential importance of this observation as a means of studying the mechanism of insulin action, it was of interest to ascertain how widespread this phenomenon might be. Accordingly, comparable studies were made with minced skeletal muscle from cats, dogs, and rabbits, using Krebs' technic, the same B.D.H. insulin as well as a highly purified insulin powder (Lilly) with an equally low Zn content, and similar solutions. As a check on technic, the experiments with minced pigeon muscle were repeated. Fed, fasted, and post-absorptive animals were used.

Results: No stimulating effects of insulin were observed on the oxygen consumption of minced muscle from cats, dogs, and rabbits under various nutritional conditions. The insulin concentrations varied from 1.5 to 70 mgm. per cent (1 mgm. = 22 units). Repetition of the experiments with pigeon breast muscle showed a consistent rise in oxygen consumption over the controls, but generally of only about 20 per cent. There was no proportional increase in this effect with varying amounts of insulin over the range indicated.

Conclusions: The in vitro effect of insulin in sustaining the respiration of pigeon breast muscle does not occur with minced muscle from dogs, cats and rabbits. The significance of this phenomenon in pigeon muscle as exemplifying the general mechanism of insulin action on carbohydrate oxidation and on the suggested "citric acid cycle" is therefore open to question. The possibility that the insulin effect on these mammalian tissues is masked by an isocaloric shift in the substrates oxidized must be borne in mind and is now under investigation.

The factors concerned in the vasoconstriction from tobacco smoking. ISRAEL

SHULMAN and MICHAEL G. MULINOS. Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York City.

The effects of cigarette smoking upon the vascular reactions of the hand

¹ This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

were studied by the methods described elsewhere, (Am. J. Physiol. **125**: 310, 1939). Seventeen normal subjects smoked standard and denicotinized cigarettes and the vascular effects were compared with the effects from 10 deep breaths.

The Hewlett inflow method and the plethysmograph showed no difference in the degree of vasoconstriction between 10 deep breaths of air and the inhalations of cigarette smoke. When inhalation is practised, the vasoconstriction was equally great with denicotinized and with standard cigarettes. There was some vasoconstriction from simple puffing. The skin temperature fell more and persisted longer from inhaling cigarette smoke than from deep breathing. Capillary observations revealed slowing or stopping of blood-flow in the tufts related to deep breathing with or without smoking.

The vasoconstriction from smoking may be due to *a*, irritation of the smoke; *b*, its nicotine content; *c*, mere inhalation. That nicotine is a minor factor is shown by the above experiments, and by observations of three smokers who smoked furiously and who showed nausea and vomiting but no vasoconstriction. It is concluded that the irritation and especially the deep breathing are responsible for the peripheral vasoconstriction from smoking.

Reflex phenomena in the delimited spinal cord of the dog. P. S. SHURRAGER (by invitation) and E. A. CULLER. University of Rochester, Rochester, N. Y.

In dogs, under complete anesthesia, the spinal cord is transected in upper lumbar region, with preservation of the principal blood-supply; the left semi-tendinosus (S-T) muscle is exposed without damage to nerve- and blood-supply, and the tendon cut near its insertion. After initial tests, the spinal roots both dorsal and ventral are cut on both sides behind the level of transection, except only the 6th dorsal and 7th ventral lumbar roots on left side and the complete third lumbar on right side. This last root is preserved to avoid damage to the blood-supply and has presumably no part in the effects observed. So complete is the root-severance that the entire cord aboral of transection can be lifted from its bed, save for blood-vessels and the four intact roots. The left hind-paw is then stimulated with a single electric shock, and the response of the exposed S-T muscle and of the whole hindlimb is observed.

After transection of cord but prior to severance of individual roots, the paw-shock elicits ordinary flexion, followed by relaxation, of S-T and other muscles, without noteworthy incident.

After severance of all roots except those stated, the response changes greatly. A single shock is followed 1, by the expected flexion-relaxation, then 2, by a strong extensor-thrust of the whole hindlimb, followed by relaxation; finally 3, by a smaller flexion-relaxation similar to 1. In five preparations the same pattern and sequence was observed, differing only in amplitude of the entire pattern and in relative magnitude of certain elements. The third phase was about one-third as large as the first, and also slowed.

The excitatory process in the isolated skeletal muscle fibre preparation. F. J. M. SICHEL (by invitation) and C. LADD PROSSER. University of Vermont College of Medicine, Clark University, and the Marine Biological Laboratory, Woods Hole, Mass.

The isometric twitch-like response of an isolated length of a muscle fibre (frog) resembles in form and time relations the isometric twitch of a whole muscle. This has led to the view that the same ultimate mechanism is involved in both cases, although the single fibre response does not usually involve the conductile mechanism.

To study the excitation of the contractile system, and the changes in its excitability following a response, pairs of stimuli were given and the peak isometric tension developed was recorded. The results of this method of stimulation show that temporal summation may occur in the isolated fibre. If the interval between two equal shocks is less than about 0.003 second the isometric tension developed is greater than twice that due to a single shock. This indicates that the summation is of a local excitatory process which reaches a maximum at or very shortly after the moment of stimulation. The intensity of this local state of excitation decreases rapidly at first, then more gradually, reaching the resting level in about 0.03 second. The results indicate further that the graded responses of the isolated fibre elicited without involving the propagated disturbance are not associated with a refractory period. The second stimulus can always contribute to the response, either by causing additional tension when the stimuli follow one another closely or by maintaining tension when the stimuli are further separated. The refractory period and the propagated disturbance are closely related and their absence allows additional stimulus above the threshold level to produce additional excitatory change and consequently a graded response.

The effect of treatment with histamine on anaphylactic shock in guinea pigs.

E. A. SMITH (introduced by G. A. Talbert). University of North Dakota, Grand Forks.

The approximate minimum lethal dose of subcutaneously administered histamine dihydrochloride in guinea pigs was found to be 0.18 mgm. of histamine base per 100 grams B. W. Treatment with histamine was begun with an initial dose of 0.0006 to 0.003 mgm. of histamine base per 100 grams B. W. and the dosage was increased 50 to 100 per cent with each injection over a period of two to three weeks.

While only 8 of 30 sensitized controls survived a shocking dose of horse serum, 55 of 60 treated animals sensitized to horse serum survived the shocking dose which was given by intravenous or intracardiac routes. The greatest degree of protection against anaphylactic shock was obtained by the administration of histamine during both pre-sensitization and post-sensitization periods. Of the 40 animals treated by this method only 4 died. A doubtful degree of protection was obtained when histamine was administered late in the post-sensitization period only.

These results indicate that in a significantly large number of cases treatment with histamine as described prevents death due to anaphylactic shock due to horse serum in guinea pigs.

The neural centers concerned in optic nystagmus. KARL U. SMITH (introduced by E. A. Culler). University of Rochester, Rochester, N. Y.

Optic nystagmus was produced in cats and guinea pigs at different velocities of movement of the visual field by rotation of a large partly-spherical drum, the inside surface of which presented a striated pattern of alternate black and white lines. The animals were restrained and placed

inside the drum so that the striations covered the entire extent of the visual field. In the cats, the nystagmic reactions of the eyes were recorded oscillographically, while in the guinea pigs, kymographic ink-recordings were made of head nystagmus. Quantitative determinations of terminal threshold velocities were made with both groups of animals. Thereafter, the effect of complete bilateral removal of the visual areas of the cortex upon the responses was investigated in the cats (6 animals). In the guinea pigs, the normal records were compared to those secured on animals after bilateral removal of the occipital areas (6 animals), hemidecortication (9 animals), hemidecortication combined with removal of the occipital area of the other hemisphere (4 animals), complete decortication (12 animals), and unilateral and bilateral lesions of the superior colliculus (10 animals).

In the guinea pig, cortical operations of the sort described above failed to produce significant modifications in the upper threshold velocities of optic head nystagmus. Operated animals all displayed nystagmic reactions within the speed range of normal guinea pigs. Similarly, destruction of both visual areas in the cat does not modify the form, magnitude, or terminal threshold speeds of the responses. Unilateral lesions in the superior colliculus impair the reactions with movement of the pattern toward the operated side, while bilateral lesions abolish or impair the nystagmus both toward the right and left.

Microscopical controls of the lesions have been carried out on the brains of the cats and of the partially decorticate guinea pigs.

*The induction of premature labor.*¹ FRANKLIN F. SNYDER. Department of Obstetrics and Gynecology, the University of Chicago, and the Chicago Lying-in Hospital, Chicago, Ill.

The present experiments are concerned with the hormonal control of parturition. An attempt was made to investigate the interruption of pregnancy before full term in rabbits by a method involving the induction of ovulation. As a result of previous work, it was decided to observe the course of pregnancy following the addition of a fresh set of corpora lutea by means of a single intravenous injection of 10 rat units of Antuitrin S, an extract of urine of pregnant women.

A series of 95 rabbits was injected at various stages during the last quarter of pregnancy: 18 animals on the 25th day after mating, 23 on the 27th day, 26 on the 28th day, 12 on the 29th day, and 16 on the 30th day. The stage of pregnancy was definitely known since the animals were mated in the laboratory, and a uniform stock was used in which gestation averages 32 days.

In the entire series of animals the outstanding result was the prompt onset of parturition at a definite interval following induction of ovulation. Of the total 95 rabbits it was found that parturition began on the second or third day following injection in 85 animals, i.e., 89 per cent. In the remaining 10 animals, i.e., 11 per cent, parturition failed to occur until long past term, usually about 15 days following injection. Of the 85 animals in which parturition began within 3 days there was complete emptying of the uterus in 75 cases while in 10 cases one part of the litter was born on the second or third day following injection and the remainder was retained in the uterus until about 15 days following injection.

¹ Aided by grant from the Committee for Research in Problems of Sex, National Research Council.

The birth of living fetuses was a striking characteristic of labor induced at various stages during the last quarter of pregnancy, and afforded evidence of the similarity of the experimentally induced labor to natural parturition at term.

The experimental production and prevention of cardiac mural thrombi in dogs. D. Y. SOLANDT, REGINALD NASSIM (by invitation) and C. H. BEST. Department of Physiology and the School of Hygiene, University of Toronto, Canada.

After tying the descending branch of the left coronary artery, 2 cc. of 5 per cent sodium ricinoleate ("Soricin") were injected into the left ventricular myocardium at the tip. Necrosis of the muscle and adjacent endocardium resulted and thrombi, adherent to the injured tissue, formed. This formation was characteristically very rapid. In some dogs large pendulous thrombi, filling the tip of the left ventricle, were found 30 minutes after the injection of the sclerosing solution. The thrombotic mass always consisted of a central white thrombus which was adherent to the injured tissue. Over this was a layer of mixed thrombus which in turn was frequently covered by a red thrombus or clot. The administration of heparin prevented the formation of such macroscopic thrombi for periods up to 24 hours. Microscopic masses of agglutinated platelets were sometimes present on the injured endocardium, but no macroscopic thrombus was seen in a large series of these heparinized animals. Efforts are being made to continue the administration of heparin for a period long enough to permit healing of the endocardium.

Influence of the hypophysis on carbohydrate metabolism. SAMUEL SOSKIN, R. LEVINE (by invitation) and W. LEHMANN (by invitation). Metabolic Laboratory of the Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago, Ill.

Using eviscerated animals, to obviate the new formation of unknown amounts of sugar by the liver during our experiments, we have determined the effects of various hormones on carbohydrate utilization by a direct balance method previously described (Am. J. Physiol. 120: 761, 1937). Our present results show that potent pituitary extracts, which maintain the blood sugar level of fasting hypophysectomized animals, have no effect upon the rate of carbohydrate utilization of eviscerated normal animals. Neither do these extracts affect the normal rate of muscle glycogen breakdown and lactic acid accumulation. The removal of the hypophysis, on the other hand, results in a significant decrease in the rate of carbohydrate utilization at any given blood sugar level, and markedly inhibits the breakdown of muscle glycogen and the accumulation of lactic acid. Comparing the normal with the hypophysectomized animal, it is clear that the hypophysis increases the rate of muscle glycogen breakdown and carbohydrate utilization. An excess of anterior pituitary hormone, above that normally present, does not cause further increase in these processes but certainly causes no decrease.

These results are in disagreement with the conception that the hypophysis normally inhibits carbohydrate utilization and exerts a "glycostatic" effect, and that the removal of the hypophysis increases the rates of glycogen breakdown and carbohydrate oxidation. This conception was based upon the higher R.Q. and the greater decrease of carbohydrate

stores in fasting *intact* hypophysectomized animals, as compared with fasting *intact* normal animals. Our present results and other data indicate that the apparent increased disappearance of sugar in fasting hypophysectomized animals is not due to increased utilization, but to a markedly diminished gluconeogenesis in the liver, which allows a depletion of the carbohydrate stores in spite of a diminished utilization. Conversely, the so-called "glycostatic" effect of pituitary extracts is due to an increased formation of carbohydrate in excess of the amount being utilized, which results in an increase in the carbohydrate stores.

Blood-hemoglobin concentration and body-size. C. R. SPEALMAN (introduced by Ernst Fischer). Department of Physiology and Pharmacology, Medical College of Virginia, Richmond.

The blood hemoglobin concentration and the body weight were determined on groups of rabbits, men, and women. Standing height was also determined on the men and women. The results are analyzed statistically. In each of the three cases the lower limit of hemoglobin concentration is nearly independent of body size; the upper limit of hemoglobin concentration varies with body size and probably passes through a maximum.

Separation of the resting and activity oxygen consumptions of frog muscle by means of sodium azide. J. N. STANNARD. Department of Physiology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

Sodium azide (NaN_3) is known to exert marked inhibitory effects on the respiration of yeast at acid pH and on indophenol oxidase preparations (Keilin, 1936).

In these experiments it has been found that sodium azide exerts no immediate inhibitory effect on the O_2 uptake of resting frog muscle over a ten-thousand fold range of concentration (10^{-5} to 10^{-1} M). No marked change in effect occurs on lowering the pH except a slightly more rapid decline in rate after the first hour. In contrast the increment in respiration caused by electrical or chemical stimulation or subcontracture doses of caffeine is completely eliminated within a few minutes by 0.002 to 0.005 M azide. At lower concentrations the inhibition of the increment is incomplete, while higher concentrations cause no decrease in rate below the resting level. Furthermore the extra O_2 uptake is inhibited even at pH 7.4 and the fractionation is not markedly altered by lowering the pH.

Since the oxidation of p-phenylene diamine is almost completely inhibited, while the increased oxygen consumption associated with the addition of autoxidizable carriers such as methylene blue and pyocyanin is not prevented, the azide is probably inactivating the cytochrome-cytochrome-oxidase system.

Cyanide inhibits both the resting and extra oxygen consumptions but with sufficiently different kinetics to indicate that the action may be on different enzyme systems.

The results cannot be explained on the basis of "unsaturation" of the enzyme surfaces with substrate since actually large quantities of oxidizable substrate were being produced by the glycogenolytic system.

It is proposed that the respiration of frog muscle can be separated into two qualitatively distinct fractions, and that only the activity respiration traverses the cytochrome-cytochrome-oxidase system.

Further studies on the ballistocardiograph (an apparatus for the recording of the heart's recoil and the blood's impacts in man). ISAAC STARR. Hartzell Department of Therapeutic Research, University of Pennsylvania, Philadelphia.

Further studies have been made on the ballistocardiograph as a mechanical instrument. Due to vibration periods existing in the human body the descending part of the ballistic curve is an unreliable index of the forces applied. The ascending part is reasonably reliable but it should be corrected for overshooting if the waves are in phase with the body's period.

Abnormal types of ballistocardiogram encountered in patients with heart disease, may be derived theoretically from abnormal curves of blood velocity in the aorta. Curves of similar abnormal types have been produced in acute animal experiments by asphyxia, by chloroform, and by injuring the right ventricular wall.

When the ballistocardiogram has an abnormal form the formula which permits calculation of cardiac output from the normal record may not be employed. However new constants for the old formula may be derived theoretically.

The old equation may be written in the following form—(Cardiac output)² = K (altitude of most conspicuous up wave + altitude of most conspicuous down wave in mm.) (aortic cross section area in cm.²) (duration of cardiac cycle in sec.) (duration of ejection in sec.). When the form is normal K = 370. For various extremely abnormal forms K has been calculated to range from 690 to 800. For intermediate forms, intermediate values should be taken.

As abnormal forms of ballistocardiogram occur infrequently, except in very sick persons, the opportunity of comparing the cardiac output calculated from the abnormal ballistocardiogram with that obtained by other methods has come infrequently. In three cases there was reasonable agreement with results obtained by ethyl iodide.

The relation of dry mouth to thirst in the human. F. R. STEGGERDA. Department of Physiology, University of Illinois, Urbana.

In a college student devoid of any active salivary glands it was found that in order to keep his mouth comfortably moistened he had to drink small quantities of water on the average of twelve to eighteen times a day. This, he claimed, was necessary to relieve a dryness in his mouth that was not akin to thirst. The record of his total fluid intake over a period of eighteen days was in no way appreciably different from that of four other apparently normal individuals.

To test whether any sensation of thirst could actually be aroused in the subject, experiments were performed on four different occasions in which 10 grams of sodium chloride in 100 cc. of water was given by mouth twelve to fourteen hours after the last meal. Hourly records of water intake, urine output, and urine chlorides were kept for a period of six hours after the beginning of the experiment. On each occasion two normal subjects were treated similarly to serve as controls. The results indicate that, along with a definite sensation of thirst, all the subjects showed an appreciable increase in fluid intake and output during the experimental period. This, it is believed, shows that the increased intake of water by an individual when thirsty is not necessarily related to a dry mouth.

The effects of acetylcholine and eserine on frog muscle. S. E. STEIMAN and ROBERT HODES (introduced by A. Rosenblueth). Department of Physiology, Harvard Medical School, Boston, Mass.

Large bull frogs were anesthetized with urethane and the responses of the gastrocnemius to maximal indirect stimulation recorded isometrically. After cannulating the tibial artery, drugs were forced in a retrograde direction into the sciatic artery and the intact circulation carried the substances to the muscle.

Lymph sac, but not arterial injection of eserine produced a 30 to 60 per cent augmentation of the response to single shocks. Responses typical of more rapid stimulation were obtained after eserine with frequencies as slow as 1 per second.

Large doses of acetylcholine (50 to 100 γ) injected during stimulation at 1 per 8 seconds cause a prolonged depression of the response and a marked contracture. After the response has returned to normal, similar or even smaller successive doses produce greater and longer-lasting depressions, while the contractures are smaller. Acetylcholine (10 to 50 γ) injected during the steady level of the response to frequencies between 16 and 150 per second causes a sharp fall in tension. At no time during the period of ultimate decline of tension did acetylcholine (10 to 400 γ) cause a rise in tension greater than that due to the contracture.

Instead of decurarizing, as it does in the mammal, acetylcholine injected into partially curarized frog muscle usually produces a further depression. In some cases decurarization was obtained by acetylcholine. Also in a few experiments the first effect of acetylcholine was to increase the response of the curarized muscle, but this was followed by a marked depression of subsequent contractions as well as by a contracture. Decurarization could be produced by a short (5 to 15 sec.) tetanus (40 to 100 per sec.) and by lymph sac injection of eserine.

The electroencephalogram in lead-poisoned cats. S. E. STEIMAN and P. O. THERMAN (introduced by H. Davis). Department of Physiology, Harvard Medical School, Boston, Mass. (Read by title.)

The EEG's of 7 cats were recorded with an oscillograph (ink-writer) before and after poisoning with lead. Records were taken from frontal and occipital areas with electrodes on the intact scalp and reference lead on the ear. Broadly tuned filters were also used for studying 8 different frequency bands between 3 and 36 cycles per second. Care was taken to obtain records while the animals were awake. After EEG's under normal conditions were recorded the cats were given lead by stomach tube. Definite symptoms of poisoning appeared in about a month. These were a loss in weight, loss of muscular tone, and inability to walk properly. During this time a second record was taken. One cat was allowed to recover for a short period, after which lead was given again. The same procedure was repeated several times and records were obtained during different stages of recovery as well as poisoning.

The EEG's thus recorded (19 in all) show significant changes in the pattern from both frontal and occipital leads. The difference between normal and abnormal pattern is clearest in the bipolar lead frontal-occipital. The changes can be classified into two groups. The first group is found in early stages of poisoning and is characterized by a decrease in amplitude of potential and a tendency to instability in the

rhythms. The second group of records corresponds to a more marked clinical picture of poisoning and is characterized by an increase in amplitude with irregular slow-wave pattern. The 6-8 per second rhythm usually seen as a definite rhythm in normal cats fluctuates here between faster and slower frequencies. During recovery the brain waves change from a high-voltage, irregular, slow-wave pattern through the low-voltage stage towards the normal record. The modifications in the EEG are characteristic of the various stages of lead-poisoning. The alterations in the EEG resemble those obtained in humans falling asleep.

Electrolytes in Thyone muscles. H. BURR STEINBACH and JACK AURINGER (by invitation). Department of Zoology, Columbia University, New York City.

The retractor muscles of *Thyone briareus* contain on the average, in milliequivalents per hundred grams, Na 17.3, K 16.9, Ca, 1.4, and Cl, 20.4. Except for a small residue Na and Cl are frequently diffusible indicating Cl or Na-spaces of about 40 per cent. Penetration of potassium and calcium into the cells (chloride-free portion of the muscle) was followed by analysing muscles soaked in modified sea water containing known concentrations of these cations, the chloride space being determined as well as the cation in question. All solutions were approximately isotonic with sea water. Curves were obtained by plotting relative weight, chloride space and cation concentration against potassium or calcium concentration of the medium.

Weight changes were relatively slight, the greatest being, on the average, a 12 per cent gain in pure KCl and a 12 per cent loss in pure CaCl_2 . Chloride space figures also show little variation from the normal, the most significant being a decrease in pure CaCl_2 that almost accounts for the loss of weight of the whole tissue in the same solution.

Assuming that chloride free space represents cell space, the figures show that K penetrates and is concentrated in the cells from all concentrations in the medium up to 0.35 normal. On the basis of concentration per unit volume of cell water, potassium is always present inside the cells in considerably higher concentration than in the medium.

Calcium behaves in a distinctly peculiar fashion. Some of the calcium of the normal muscle is indiffusible and hence with very low concentrations in the medium Ca may be said to be concentrated in the cells. As the calcium of the medium is increased, between 0.0 and 0.1 normal, cell calcium also increases. Higher concentrations of calcium in the medium result in a progressive loss in cell calcium until in muscles treated with 0.2 normal CaCl_2 in sea water the calcium content of the cells is about that found in the cells of muscles soaked in calcium-free solutions.

Injury potentials of giant axons of the squid. H. BURR STEINBACH. Department of Zoology, Columbia University, New York City. (Read by title.)

Injury potentials developed by single giant axons of the squid were measured between an injured end immersed in isotonic KCl and an uninjured portion bathed in sea water, isotonic KCl, isotonic (approximately) CaCl_2 or dilutions of these with isotonic sucrose solution. The average P.D. between the injured end in KCl and an uninjured portion in normal sea water was 39 millivolts. Considerable variation was noted, poten-

tials as high as 72 millivolts being observed in some cases. The injured end in KCl was always negative to the uninjured region except when isotonic KCl was applied which nearly abolished any potential difference. The P.D./log concentration curve for KCl was nearly linear with average values of 0.5 mv. in 0.52 normal KCl to 49 mv. in 0.005 normal KCl. For sea water the curve was slightly concave (average values: 33 mv. in sea water to 60 mv. in 0.001 sea water). With CaCl_2 the curve is markedly concave, average terminal values being 37 mv. in 0.54 normal CaCl_2 and 48 mv. in 0.0054 normal.

The concentration effects are not strictly reversible, hence precise evaluation of the data is difficult. However, the effect of sea water and CaCl_2 concentration appears to be due to simple diffusion effects with relative ionic mobilities unaltered from the normal. Potassium is unique in being the only ion apparently penetrating the axon much faster than Cl.

A new concept regarding the mechanism of clotting and the control of hemorrhage. ARTHUR STEINBERG and WILLIAM R. BROWN (introduced by A. C. Ivy). Research Foundation in the Kensington Hospital for Women, Philadelphia, Pa.

Extracts have been prepared from certain plants which have been found to accelerate markedly and rapidly the rate of coagulation of the blood. The best plant sources have been found to be shepherd's purse, wood sorrel, beets, oxalis, citrus fruits, rhubarb, euphorbia, etc.

This extract has been biologically assayed in rabbits and a unit has been devised which is defined as the minimum amount of material which will reduce the coagulation time of a five-pound rabbit 50 per cent fifteen minutes after an intravenous injection. This extract was found to be non-toxic in rats, guinea pigs and rabbits, contained no alkaloids or proteins, and could be administered intravenously or intramuscularly without ill effects. It was subsequently utilized in over five hundred patients manifesting a hemorrhagic tendency.

A similar extract was also prepared from placenta, cord blood, spleen, liver, bile, etc. which also possessed marked hemostatic properties.

Colorless, monoclinic crystals possessing most of the clotting power of the extract were isolated in pure form with a melting point of 101°C . This substance was identified as oxalic acid. Solutions of pure commercial oxalic acid were prepared in the same concentration as present in the extract and were found to markedly and rapidly reduce the coagulation time after intravenous injection into rabbits.

A titrametric test for oxalic acid in the blood was devised, based on a modification of Sasuki's method (Japanese Journal of Medical Science, June 1934). Normal values for humans were established at 5.5 to 7.5 mgm. per 100 cc. and in rabbits at 4.5 to 6.0 mgm. per 100 cc. Elevations in clotting time were attended by a fall in the blood oxalic acid, while reductions in clotting time conversely resulted in a rise in the oxalic acid content in the blood. Cord blood was found to contain as high as 17 mgm. per 100 cc. while maternal blood, postpartum, also showed a considerable elevation in the blood oxalic acid. In both instances the clotting time was appreciably reduced.

These findings led us to investigate other polybasic acids, particularly the saturated di-carboxylic acids of the formula $\text{C}_n\text{H}_{2n}-2\text{O}_4$.

The level of pressure in the pulmonary artery of the dog with renal hypertension. F. S. STEINITZ (by invitation) and L. N. KATZ. Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

Renal ischemia is known to result in hypertension in the systemic circuit, but its action on the pulmonary circuit has not yet been reported. We have investigated the pulmonary pressure in normotensive and hypertensive dogs. Two types of experiments were performed. In both, pulmonary and femoral arterial pressures were recorded with Hamilton manometers before and at various periods after hypertension was established.

In the first type the pulmonary arterial pressure was recorded by means of a temporary opening in the third or fourth left intercostal space, the dog being anesthetized with nembutal and under artificial respiration during the procedure. After the readings were obtained, the chest was reclosed and the pneumothorax relieved.

In the second type, a silver London cannula was attached to the wall of the pulmonary artery and brought under the skin. After this had healed, the dogs were trained so that the pressure readings could be made without anesthesia.

| DOG | CONTROL ARTERIAL PRESSURES | | AFTER PRODUCTION OF RENAL ISCHEMIA ARTERIAL PRESSURES | | |
|-----|----------------------------|-----------|--|---------|-----------|
| | Femoral | Pulmonary | Days | Femoral | Pulmonary |
| 1* | 175/100 | 30/15 | 9 | 225/125 | 25/15 |
| | | | 99 | 195/135 | 21/8 |
| 2* | 175/105 | 25/16 | 26 | 200/130 | 27/13 |
| 3* | 150/85 | 25/15 | 11 | 230/125 | 21/7 |

* Experiments 1 and 2 were of the first type described while experiment 3 was of the second type. Pressures are in millimeters of mercury. We found that the normal control pulmonary pressures in the open and closed chested dogs were of the same order, viz: average of 4 of former 27/15 and 4 of latter 26/15 mm. Hg.

Our results show clearly that systemic hypertension of renal origin is not accompanied by pulmonary hypertension. Since the minute volume output of the heart and the circulating blood volume is not significantly altered in renal hypertension, these results indicate that the chemical mediator of renal hypertension apparently does not act on the pulmonary vessels.

Cord potentials in spinal shock: single volleys. WINIFRED B. STEWART (by invitation), G. P. MCCOUCH and JOSEPH HUGHES. Department of Physiology, University of Pennsylvania and Pennsylvania Hospital for Mental and Nervous Diseases, Philadelphia.

In the cat in which internuncial potentials are recorded from the dorsal surface of the cord in the decerebrate preparation, the positive wave, which follows the negative in the decerebrate, is temporarily eliminated by transection of the cord. It may return within two hours. In the spinal dog and the spinal macaque monkey we have observed positivity only in chronic preparations. In confirmation of Fulton and Sherrington (J. Physiol. 75: 17, 1932) we find that cat and dog yield ratios between the ipsilateral flexor reflex to a single maximal volley and the maximal

neuromyal twitch far larger than those of the monkey in which we have obtained myographic records of reflex response only in chronic preparations. Yet, the internuncial potential is present even in the acute monkey as early as we have attempted to record it. The threshold of the internuncial potential may approximate the A fiber threshold of the nerve stimulated in the cat (confirming Odoriz and Toennies—Toennies, *J. Neurophysiol.* 1: 378, 1938) and in the dog within an hour, in the monkey within 5 hours after transection. The reflex threshold may reach identity with the internuncial threshold in the dog immediately after transection and the two values continue to fall together until they approximate that of the stimulated nerve. Hence, in the dog, spinal shock is as intense in the internuncials as in the motoneurons. Although in many cats and dogs the threshold for the reflex is significantly higher than that for the internuncial potential, this discrepancy obtains in chronic as frequently as in acute preparations. In the monkey on the contrary, in spite of the presence of a large internuncial potential, we have never obtained a reflex response of semitendinosus in an acutely spinal preparation. The gulf that separates the monkey from the cat and the dog is due to shock to the motoneurons in the monkey out of all proportion to that obtaining in the other species.

A study of the pharmacological effects of certain saturated fluorocarbons.

H. C. STRUCK and E. B. PLATTNER (introduced by C. I. Reed). Department of Physiology, College of Medicine, University of Illinois, Chicago.

The completely substituted, saturated fluorocarbons containing from one to six carbon atoms have recently been prepared by J. H. Simons. These compounds have certain chemical and physical properties which suggested the possibility that they might prove useful as general anesthetics. They are chemically inert, slightly soluble in water, and readily soluble in fats and fat solvents. Through C_6F_{12} they are colorless and odorless gases or liquids boiling at or below body temperature.

In a specially designed apparatus mice were exposed to various concentrations of certain of these substances in air or oxygen. The compounds had a marked depressing effect on respiration and on the central nervous system, but in no case did they produce anesthesia. The gases were highly toxic in concentrations sufficient to produce depression, causing death of all animals.

Histological examination of tissues revealed marked effects on lungs, liver, heart, and brain sufficient to cause death. The changes consisted, in general, of marked congestion, edema, some hemorrhages. All findings were consistent with pathology due to irritating gases. It is impossible to state whether these effects are due to chemical action of the gases on the tissues or to simple physical causes. In view of the inert nature of the compounds, the latter possibility appears to be the more reasonable.

Further experiments are in progress, and studies on other fluorine compounds will be made as they are prepared.

Salivary conditioned reflexes in swine. GEORGE F. SUTHERLAND (introduced by H. S. Liddell). Department of Physiology, Cornell University, Medical College, Ithaca, N. Y.

Swine may easily be handled on a leash if training is begun as soon as they are weaned. A parotid fistula must be made as in the dog. The

experiments are conducted in a stout pen, 3 feet wide and 5 feet long. Hog fencing used on one side allows observation. A means must be provided for delivering to the animal, at appropriate times, a little dog biscuit, weighing about 10 grams (commercially available). A small cup cemented over the fistula previously made, collects the saliva and transmits it to the recording apparatus. One Chester White and one Duroch Jersey were used in these experiments. Positive and negative salivary conditioned reflexes were established to different tones in these animals by employing the usual Pavlov procedure as in dogs.

The absence of functional pressor activity in the carotid sinus. H. MORROW SWEENEY (introduced by H. S. Mayerson). Department of Physiology, University of South Dakota, School of Medical Sciences, Vermillion.

The carotid sinuses of large, anesthetized dogs were dissected free and their activity determined. The animals were then slowly bled until the direct femoral arterial blood pressure was permanently lowered to shock level. The effect on the blood pressure resulting from rapid denervation by mechanical stripping and painting with phenol was then noted.

In ten of the sixteen experiments the blood pressure rose gradually but appreciably, an average of 30 mm. Hg, during the next several minutes following the denervation; while in the other six no change took place. The change in the former group indicated that depressor impulses were still being initiated in the sinus until denervation, while in the latter group no effective impulses were being initiated. In no case was there a sufficient drop in pressure to indicate that a pressor activity had been destroyed by the denervation.

The effects of magnesium perfusion and production of graded responses in the turtle heart. H. L. TERRY (by invitation) and HAROLD N. ETS. Loyola Medical School, Chicago, Ill.

Turtle hearts were perfused in situ with a solution containing sodium chloride 0.7 per cent, potassium chloride 0.019 per cent, calcium chloride 0.021 per cent and magnesium chloride 0.009 per cent. Normal, steady contractions of ventricle and atria have been recorded during at least eight hours of perfusion.

Moderate increases of the magnesium chloride in the perfusate (with decrease of sodium chloride to maintain isotonicity) affected different regions and functions of the heart to an unequal extent; while greater increases of magnesium caused impairment of all functions with stoppage and marked dilatation.

During the first few minutes of perfusion with a sixty-fold increase of the magnesium content, the ventricular amplitude progressively diminishes, developing into sudden standstill with relaxation greater than the normal diastole. The atrial beat continues for some time, slow and rhythmical, but with increased amplitude. Gradually the slowing becomes more pronounced and stoppage occurs suddenly in diastole.

Electrical stimulation of the arrested heart using a platinum electrode (one pole in the ventricle and the other in the atrium) with either condenser discharge or galvanic current shows additional magnesium effects. Immediately after the ventricle has stopped, graded contractions are obtained which are proportional to the strength of stimulus. With

continued perfusion the threshold rises until no contraction occurs even with strong stimuli.

After the atria have stopped beating, they respond in an all or none manner for several hours, but with continued magnesium perfusion their responses become graded and finally the threshold rises to non-irritability.

Immediate recovery of the magnesium inhibited hearts occurs with change of the perfusate to the normal balanced salt solution, even though magnesium standstill has lasted for over six hours.

Microelectrode records from the superior cervical ganglion. P. O. THERMAN¹ (by invitation) and A. FORBES. Department of Physiology, Harvard Medical School, Boston, Mass.

The electric response of the cat's cervical sympathetic ganglion to pre-ganglionic stimuli has been recorded alternately with a microelectrode consisting of a pipette drawn to a terminal aperture of between 12 and 40 μ , and a chlorided silver wire on the surface of the ganglion, using a silver-wire electrode on the postganglionic nerve trunk as a common lead. The leads were connected to a cathode-ray recording system and the responses photographed. The records thus obtained with microelectrodes were compared with corresponding standard ganglionic records (silver wire on surface).

When the microelectrode is on the surface of the ganglion records have been found almost identical with the standard ganglionic response,—a smooth curve which barely indicates the M1, M2 and M3 groups of elements. As the microelectrode penetrates the ganglion the record is split up into separate negative spikes whose duration is from 1.5 to 3 msec. The greatest difference between the standard ganglionic potentials and those obtained by microelectrodes is seen when the microelectrode is inserted deeply into the ganglion. A change of less than 0.1 mm. suffices to change the pattern. This suggests sharply localized active regions showing small differences in latencies.

The polarity and duration of the component spikes resemble those found in the hippocampus by Renshaw *et al.* (Am. J. Physiol. **123**:169, 1938). The observations support the suggestion of previous workers that the ganglionic response is an integration of more spikes than those hitherto identified.

Inhibition of gastric peristalsis due to amino acids in the small intestine.

J. EARL THOMAS. Jefferson Medical College, Philadelphia, Pa.

The inhibitory effect on antral peristalsis of placing various amino acids in the upper small intestine was investigated in dogs having cannulated gastric and duodenal fistulas. The insoluble amino acids were made up in alkaline solutions which were neutralized just before being injected. Care was taken to avoid making the solutions hypertonic.

Little inhibitory effect was obtained with arginine HCl, cystine, or a mixture of diamino acids and cystine prepared by precipitation with phosphotungstic acid from a casein hydrolysate. Glutamic and aspartic acids were inactive in solutions neutralized with sodium hydroxide but caused definite inhibition in acid solutions (pH 3.0-4.0). The inhibition caused by these solutions was not due to hydrogen ions because phosphate buffered saline at the same pH had no inhibitory effect.

¹ Fellow of the Rockefeller Foundation.

Glycine, valine, leucine, tyrosine and amino acid mixtures containing these and other monoamino-monocarboxyl acids caused gastric inhibition in neutral solution; with the exception of glycine, their effects were not increased by acidification to pH 3.0. Glycine had a feeble effect in neutral solution which was moderately increased by addition of acid. Alkaline solutions of the monoamino-monocarboxylic acids caused inhibition only after a considerable delay regarded as sufficient for neutralization of the solution in the intestine.

Among the effective amino acids investigated the inhibitory activity increased with increasing molecular weight. However, among the inactive amino acids were some with a high molecular weight.

It is tentatively concluded that the free monoamino acids (but not their sodium salts) cause inhibition of gastric peristalsis from the intestine; therefore the action is probably contingent upon the presence of a free carboxyl group. The relative inactivity of the diamino acids is unexplained but it may be related to the suppression of some of the characteristic properties of the carboxyl group.

*The effect of hypophysectomy on the hypercholesterolemia of thyroidectomized dogs.*¹ K. W. THOMPSON² (by invitation), C. N. H. LONG and B. F. LYONS (by invitation). Department of Surgery and the Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven, Conn.

This report presents data which indicate that the pituitary gland plays a rôle in the production of the hypercholesterolemia of myxoedema.

Total thyroidectomy normally is followed by a state of surgical myxoedema, characterized by a depression of the basal metabolic rate, marked elevation of the value of the plasma cholesterol, and cytological changes in the anterior pituitary. In hypopituitarism, on the contrary, with an equal degree of thyroid inactivity, the cholesterol value of the blood plasma is normal.

The plan of the experiments was as follows: 1, hypercholesterolemia was produced in male dogs by total thyroidectomy; 2, hypophysectomy was then performed and the changes in the values of the plasma cholesterol were observed; 3, certain attempts were made to elevate the value of cholesterol in the plasma by injecting pituitary extracts.

Methods: The cholesterol value was determined by the method of Bloor. Repeated examinations of the same sample of blood varied approximately ± 5 per cent. The surgical procedures were carried out under nembutal anesthesia. The thyroidectomy was performed in such a manner as to preserve at least one parathyroid. The hypophysectomies were uneventfully accomplished via the transbuccal approach.

Results: Thyroidectomy was followed by a marked elevation of the plasma cholesterol value which, after hypophysectomy, fell to normal at the rate of approximately 100 mgm. per cent per week. In one animal the cholesterol fell to 65 mgm. per cent, and this was associated with a marked fall of the serum calcium with the development of severe clinical tetany. Administration of calcium brought about a rapid clinical improvement and a restoration of the plasma cholesterol to normal.

¹ This work was assisted by a grant from the Josiah Macy, Jr. Foundation.

² Alexander Brown Coxé Fellow 1937-1938.

The injection of pituitary extract has caused a marked elevation of the cholesterol in normal dogs, but in one dog this was not maintained following hypophysectomy, even though the injections were continued. These animals had potent thyrotropic antihormone in their sera, and in their pituitaries there were the cellular changes characteristic of thyroidectomy.

The experiments indicate that a defect in the balance of the endocrine glands, in which the hypophysis again plays a dominant rôle is the cause of the hypercholesterolemia of myxoedema.

The effects of anoxia on urine flow from normal and denervated kidneys in dogs with and without adrenals. LOUIS A. TOTH (introduced by Henry Laurens). Laboratory of Physiology, Tulane University School of Medicine, New Orleans, La. (Read by title.)

Fifteen experiments were performed on 13 anesthetized (Dial) dogs with one kidney acutely denervated. The adrenal secretion was prevented from getting into the circulation by occluding the veins approaching and leaving the glands. Anoxia was induced by the inhalation, through the cannulated trachea, of gas mixtures varying in oxygen concentration from 6.3 to 9.1 per cent. Urine rates were recorded continuously from cannulated ureters. The urinary response of the normal kidney to anoxia was the oliguria (in 9 of 12 experiments) that usually results under these experimental conditions (Toth. *Am. J. Physiol.* **119**: 127, 1937). This oliguria was independent of changes in the carotid blood pressure. In contrast to this the response of the denervated kidney to anoxia varied directly with changes in the carotid blood pressure (in 12 of 15 experiments). The elimination of the adrenal glands did not alter the responses of the normal and denervated kidneys to anoxia. Apparently the nervous control is one of the primary factors regulating urine excretion during anoxia.

Curare-like action of Erythrina alkaloids. KLAUS UNNA (introduced by H. Molitor). Merck Institute of Therapeutic Research, Rahway, N. J. Several new alkaloids isolated in pure form from various species of *Erythrina* have been studied for their curare-like action in frogs, mice, rabbits, and cats.

In frogs doses from 2 to 10 mgm. per kgm. caused typical curare-like paralysis of very short duration. Higher doses caused paralysis lasting up to four days. Mice injected subcutaneously developed few clonic convulsions, and death resulted from paralysis of the respiratory muscles. In cats and rabbits, as shown by the electromyogram, the skeletal muscles were paralyzed before the diaphragm. As in frogs the paralysis in cats was of remarkably short duration. Under artificial respiration cats given a paralyzing dose intravenously fully recovered in less than one hour. Subcutaneous injection of certain of the longer acting alkaloids made it possible to keep cats motionless and nearly completely paralyzed for one hour without affecting the diaphragmatic respiration.

Some of the alkaloids injected intravenously produced a transient fall in blood pressure due to vagus stimulation.

In contrast to curare they seem to be rapidly metabolized; no trace was found in the urine.

The investigated alkaloids differed in their effective doses, in the duration of their action, and in their effects on the circulation. As with

curare, physostigmine or physostigmine-like substances proved to be highly effective antidotes.

The distribution of lipoids in the genital tract of the monkey at different stages of the menstrual cycle. H. B. VAN DYKE and G. CHEN (by invitation).

Department of Pharmacology, Peiping Union Medical College, and the Squibb Institute for Medical Research, New Brunswick, N. J.

Using preliminary techniques similar to those used in their first report (Am. J. Anat. **58**: 473, 1936), the authors investigated quantitatively the distribution of neutral fat, phosphatide, and free and esterified cholesterol in the various mucosae and in the musculature of the body and cervix of the uterus of *Macaca mulatta* in relation to the menstrual cycle. The tissues of a few ovariectomized monkeys after the injection of oestradiol benzoate or of oestradiol benzoate followed by progesterone also were analyzed. All the analyses were performed by the gasometric techniques of Van Slyke and his colleagues. The concentration of lipoids in the plasma and in the erythrocytes was estimated in a number of animals.

Often variations were so great that the differences between means were not significant. However, significant differences were found in several constituents. (All remarks refer to the concentration of lipoids.) Total lipoids in the endometrium were increased in the luteal and menstrual phases in comparison with the follicular phase. This also was found to be true of total cholesterol; it could not always be shown that this was due to increases in both free and esterified cholesterol. The mucous membrane of the cervix contained more total cholesterol during the luteal than during the follicular phase. The mucous membrane of the vagina contained a higher concentration of all the lipoids in the luteal stage than in any other stage of the menstrual cycle. No cyclic changes in any of the lipoids of the musculature were encountered.

The effect of enteral absorption of fluids on the recovery of the blood pressure after severe hemorrhage. EDWARD J. VAN LIERE and DAVID NORTHUP.

Department of Physiology, West Virginia University, Morgantown.

Blood pressure tracings from the carotid artery were obtained from dogs under barbitol anesthesia. They were then hemorrhaged 3.2 per cent of their body weight. The blood pressure tracings were recorded during the period of hemorrhage and one and a half hour subsequently. The small intestines were exposed and washed out with isotonic glucose solution. A loop was then prepared which embodied the jejunum and ileum. Both ends were tied off. Immediately after the hemorrhage the effect of absorption from the intestine of water, physiological salt solution and dog serum was studied. Groups of ten animals were used for each substance. A control group was also used, that is, the animals were treated like the others except that nothing was placed in the intestine.

It was found that the absorption of water or physiological salt solution did not aid in the recovery of the blood pressure. The group of animals absorbing dog serum showed a somewhat higher blood pressure at the end of an hour and a half than did the controls. The difference, however, was not statistically significant.

Amounts of the various fluids were absorbed which if they had been given intravenously would have been of distinct aid in elevating the blood pressure after a severe hemorrhage. It is concluded, therefore, that oral

administration of fluids during the acute phase of recovery from severe hemorrhage is not of great assistance to the organism as far as immediately aiding the recovery of normal blood pressure.

The non-specificity of suspensions of sodium xanthine in protecting the liver against injury by chloroform, and the probable cause of its action. H. M. VARS, I. S. RAVDIN and SAMUEL GOLDSCHMIDT. Harrison Department of Surgical Research and the Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia.

The finding of Forbes and Neale, their co-workers, and others that a suspension of sodium xanthine injected previous to exposure to chloroform protects the livers of rats against injury has been confirmed. The investigations have been extended with the view of testing the specificity of the effect and its probable mode of action.

It has been found that subcutaneous injections of a solution of sodium ricinoleate twenty-four hours before one hour of chloroform anesthesia resulted in complete protection of rats from hepatic injury. Injection of colloidal carbon under the same condition also was partially effective.

It would appear that substances which produce an inflammatory process or a subcutaneous abscess protect the liver equally as well as sodium xanthine and in the case of sodium ricinoleate the protection was absolute under the conditions of our experiments.

The explanation adopted as a working hypothesis which is now under investigation, is that first suggested by Daft, Robscheit-Robbins, and Whipple. In short, the protective action is due to "protein-split" products made available to the liver by the increased protein catabolism in the body in the presence of abscesses. This hypothesis conforms with previous findings that high protein diets are very protective against injury to the liver by chloroform.

A method for the bioassay of renin. G. E. WAKERLIN and G. R. CHOBOT (by invitation). Department of Physiology, College of Medicine, University of Illinois, Chicago.

Methods for the bioassay of renin have included blood pressure determinations on the rat, rabbit, cat, and dog, as well as perfusion of the tail and of the leg of the dog. Of these the pressor response of the blood pressure of the dog to renin is probably the most satisfactory assay criterion. However, in our experience only one-third of 30 dogs proved to be satisfactory assay subjects. The unsatisfactory animals were about equally divided into three groups: 1, no pressor response to a standard intravenous dose of 0.5 gram of kidney equivalent per kilo of assay animal; 2, a pressor response to the first injection and none to subsequent injections, frequently with a failure of the blood pressure to return to its original level; and 3, a diminishing response to successive injections. When renin was prepared from beef or hog instead of dog kidneys, the assay results were even less satisfactory.

On the other hand, two-thirds of 60 dogs bilaterally nephrectomized one to three hours before injections of renin were begun, proved to be satisfactory assay subjects. Furthermore, a study of the reactivity to renin before and after bilateral nephrectomy in 15 dogs showed an increased response one to three hours following removal of the kidneys in

the 10 dogs of this group which gave a poor response to renin prior to nephrectomy.

On the basis of these findings we now use the following bioassay procedure for renin. The dog is given a sedative dose (100 mgm. per kilo) of sodium barbital subcutaneously. Under temporary light ether anesthesia one femoral artery is cannulated for mean blood pressure readings, the opposing femoral vein is prepared for injections, and through lumbar incisions extraperitoneal bilateral nephrectomy is performed. Three hours later the animal is considered ready for assay. Injections of renin preparations are made at intervals of one-half hour. We have found at least three out of four dogs to give satisfactory results with this method.

The effect of the reticulocytogenic urine principle administered orally in pernicious anemia. G. E. WAKERLIN. Department of Physiology, College of Medicine, University of Illinois, Chicago. (Read by title.)

Normal human urine contains a principle which is reticulocytogenic for the pigeon, rat, and guinea pig, as is the antipernicious anemia liver principle. A urine extract prepared by a method basically that employed in the making of liver extracts is likewise reticulocytogenic for the pigeon. However, this urine extract is ineffective intramuscularly in pernicious anemia, thus proving the nonidentity of the reticulocytogenic urinary substance and the antipernicious anemia liver principle. However, the possibility still remained that the urine extract might be effective by mouth in pernicious anemia. This possibility was perhaps strengthened by the fact that kidney extract prepared by a method which yields potent liver extracts, although reticulocytogenic for the pigeon is ineffective intramuscularly in pernicious anemia, whereas kidney by mouth is therapeutically active. Hence we administered urine extract to three suitable, untreated pernicious anemia patients by mouth in a dosage of two capsules per day for ten days, each capsule being derived from 300 cc. of normal human urine. The urine extract was completely inactive by this route, proving the nonidentity of the urine and renal principles. Obviously more work must be done in order to clarify the physiological interrelationships, if any, of these hepatic, renal, and urinary principles.

The effect of normal and renal hypertensive dog plasmas on surviving arterial rings. G. E. WAKERLIN and M. YANOWITZ (by invitation). Department of Physiology, College of Medicine, University of Illinois, Chicago. (Read by title.)

At the present time there is inconclusive evidence both for and against the presence of a pathogenetic pressor substance in the blood of dogs rendered hypertensive by the technique of Goldblatt. We have studied this question by determining the effect of citrated plasmas from six normal and six renal hypertensive dogs on the tonus of surviving beef arterial rings. Blood was secured from the femoral artery at weekly or longer intervals. Three to six plasma specimens from each dog were studied. The plasmas of three of the dogs were tested before and after partial occlusion of the renal arteries. The vasoconstrictor effects of the several plasma specimens from the same dog proved to be fairly constant. When the plasmas were allowed to stand in the ice box for 24 hours, the vasoconstrictor effect was increased. There was no correlation between the

tone augmenting action of the plasmas on the arterial rings and the blood pressure levels of the dogs. There were no significant differences between the vasoconstrictor effects of the normal and the renal hypertensive plasmas, nor between the plasmas before and after the production of experimental renal hypertension. These results speak against the presence of a pathogenetic pressor substance in the blood of Goldblatt dogs but by no means rule out this possibility.

An experiment in human vitamin A-deficiency. GEORGE WALD and DAVID STEVEN¹ (by invitation). Biological Laboratories of Harvard University, Cambridge, Mass.

A second experiment in human vitamin A-deficiency has been performed in this laboratory, with methods previously described (Wald, Jeghers and Arminio. *Am. J. Physiol.* **123**: 732, 1938). The dark adaptation of the subject (D. S.) was measured at regular intervals during a period of vitamin A saturation, in which the normal diet was supplemented with 51,000 units (U.S.P.XI) daily of vitamin A; and during a subsequent 35 days on a diet containing only 100-200 units daily of vitamin A, but supplemented with optimal amounts of vitamins B₁, B₂, C, D, calcium, phosphorus and iron.

On the vitamin A-deficient diet, the minimal dark adapted thresholds of both cones and rods rose regularly. The ascent of log threshold, plotted against time, was linear; and for both rods and cones the lines which describe this rise originate on the first day of the deficiency. The log minimal threshold rose 0.012 per day in the cones and 0.025 per day in the rods. Corresponding values for the previous subject (Arminio) had been about twice as great: 0.022 in the cones and 0.054 in the rods. This is the only significant difference found in the two experiments.

The threshold of rod vision appears to be inversely proportional to the retinal content of rhodopsin. A linear rise in log threshold probably corresponds, therefore, to a simple logarithmic fall in rhodopsin concentration. If this in turn is assumed to be an index of the general vitamin A level, the latter also decreases logarithmically on the deficient diet; that is, each day a constant fraction of the vitamin A reserve is lost.

On the 35th day of the deficiency the standard dark adaptation procedure showed the minimal threshold of the cones to be 3.4 times, that of the rods 9.1 times above normal. 20,000 units of carotene in oil (Smaco) were administered orally in gelatine capsules. For 14 minutes the dark adapted threshold of the rods remained unchanged; then it broke sharply, and in 49 minutes had descended to the normal level. Immediate repetition of the standard procedure showed both cone and rod thresholds to have entered the normal range.

Localization of cochlear potentials. EDWARD M. WALZL and JOHN E. BORDLEY (introduced by C. L. Gemmill). Otological Research Laboratory, Johns Hopkins University, Baltimore, Md.

The problem of tonal localization of cochlear response was studied by a new technique, regional detachment of the spiral ligament from the bone. Carefully done, this procedure causes relaxation of tension on the basilar membrane and traumatic injury of the organ of Corti, limited to the region

¹ Henry Fellow.

of detachment, but no tearing of Reissner's or the basilar membrane (histologically confirmed).

Small detachments about 3 mm. from the basal end of the cat's cochlea (12 experiments) impaired thresholds of cochlear potentials for high frequencies (2048 to at least 14,263 cycles per second). Only two had an initial impairment for lower frequencies. Slight extensions of the detached area greatly increased the impairments for high frequencies and caused slight but definite impairments for the lower frequencies. Impairments of 50 to 60 decibels (db.) for 4096 were thus obtained with losses of only about 10 db. for 256, 512 and 1024.

When small detachments were made 5 mm. instead of 3 mm. from the basal end, 1024 instead of 2048 was the lowest frequency to show an initial impairment (6 of 8 experiments). Otherwise, similar effects were produced by lesions at these levels.

Detachment at the 7 mm. level, however, caused an almost uniform impairment (8 experiments) for all frequencies from 256 upwards (no lower tones were tested). This level is only about one-third the total distance, 20 mm., from base to apex. Basal-turn detachments in the guinea pig gave effects similar to those in the cat.

Detachments of the spiral ligament in the second turn, studied only in the guinea pig, impaired responses slightly (10 to 15 db.) for 1024 and lower tones. With the electrode either on the apex or near the round window, threshold response for 4096 was not impaired, but response to stimulation with higher intensities showed marked impairment with the apex lead.

These observations on cochlear potentials and the studies of human hearing made in this laboratory both indicate that tonal localization in the cochlea is far less sharp than usually assumed, also that there is more localization for high than for low tones. This agreement strengthens the view that cochlear potentials arise in the organ of Corti.

Effect of chemicals on cochlear potentials. EDWARD M. WALZL (introduced by C. L. Gemmill). Otological Research Laboratory, Johns Hopkins University, Baltimore, Md. (Read by title.)

The effect of chemicals on cochlear potentials in cats was studied in an attempt to throw more light on the relation of these potentials to hearing, and to determine the mechanism and structures involved in their production.

Application of crystals of NaCl or KCl to the round window membrane or to the operatively exposed endosteum of the basal coil caused initially a marked impairment for high tones, while low tones were the first to be affected by placing crystals of these salts into the opened apex. With both of these methods, as the chemicals spread, other tones became impaired.

To replace all the cochlear *perilymph* with solutions of known composition, holes were drilled through the bony wall of the scala tympani and of the scala vestibuli of the basal turn, and the solution was introduced by a specially designed apparatus. Fifteen control experiments in which the cochlea was perfused with cerebro-spinal fluid showed that the operative procedures involved do not affect cochlear potentials. The solutions studied were: isotonic NaCl, KCl, CaCl₂, dextrose and sucrose; hypo- and hypertonic cerebro-spinal fluid; and cerebro-spinal fluid with increased

or decreased pH. With all of these solutions the impairment of cochlear potentials which resulted was equal for all tones. There was, however, a great difference in the time before onset of any impairment and in the total impairment caused by the different solutions.

Conclusions: 1, the effect of a salt on cochlear potentials is due to a chemical action and not to osmotic damage; 2, the cochlear response to high tones is localized toward the basal end of the cochlea and that for low tones, at least to some extent, toward the apex; 3, cochlear potentials are neither streaming nor concentration potentials across either of the membranes that separate the scala media from the perilymphatic scalae; 4, the structures giving rise to cochlear potentials are in the scala media, and are probably the hair cells of the organ of Corti.

*The metabolism of rabbit bone marrow in serum.*¹ CHARLES O. WARREN, JR. (introduced by J. C. Hinsey). Department of Physiology, Cornell University Medical College, New York City.

In an earlier publication, the author has described the oxygen consumption of rabbit bone marrow (in Ringer's solution) in relation to its morphology. The present communication is an extension of these studies in which measurements are made of both the rates of oxygen consumption and glycolysis of slices of rabbit bone marrow suspended in sera of various kinds. A method is described for the use of neutralized serum as the suspension medium in the ordinary Warburg manometers and the results so obtained are compared with similar experiments using Ringer's solution and also untreated serum by a combination of the Dixon-Keilin and Dickens-Greville methods. The glycolysis measurements are checked with chemical determinations of the lactic acid produced. It is found that the rates of both oxygen consumption and glycolysis are much higher in serum (whether horse serum or rabbit serum and by either of the methods described) than in Ringer's solution. Moreover, in serum, these metabolic rates are maintained constant for periods as long as five hours, whereas in Ringer's solution there is a considerable falling-off of the metabolism during this time. It is concluded that in the case of rabbit bone marrow, rabbit or horse serum, whether neutralized or unaltered, constitutes a much more physiological medium than Ringer's solution and that it can be used in the ordinary Warburg manometers by the method described.

The thickness of the limiting envelope of mammalian erythrocytes. DAVID F. WAUGH (by invitation) and FRANCIS O. SCHMITT. Washington University, St. Louis, Mo.

A few ml of a dilute suspension of erythrocytes are placed on a prepared slide. After a few minutes the non-adherent cells are washed off with isotonic saline and the slide is immersed in M/10,000 buffer solution. After hemolysis the stromata are dried and the thickness of the double membranes determined by an optical method (see demonstration abstract by Schmitt and Waugh).

For the three species so far investigated the maximum thicknesses are: for ox, 210 AU (at pH 5.5), for rabbit, 173 AU (at pH 6.2), for rat 140 AU (at pH 5.5). The shapes of the thickness-pH curves varies strikingly between the species, the relative resistance to the destructive effect of pH's more alkaline than that which leaves the membrane with maximal

¹ Supported by a grant from the Committee on Scientific Research of the American Medical Association.

thickness diminishing in the order ox, rabbit, rat. The presence of isotonic salt is presumably necessary for the stability of the membrane at blood pH. Allowing the cells to remain in the hemolyzing solution for more than a few minutes, even at optimal pH, results in loss of material from the membrane (as much as 20 per cent in 5 minutes).

Extraction of the hemolyzed membranes with lipid solvents reduces the thickness, the remaining residue being presumably protein. In the case of rabbit such leaching reduces the thickness at pH of maximal thickness from 173 to 60 AU. If it be assumed that this treatment extracts only lipide the ratio of lipide and protein partial volumes is approximately 1.9, the ratio of relative weights approximately 1.2. These ratios agree with estimates based on other data.

While these experiments indicate the thickness, volume, approximate weight, and relative composition of the dried membranes, they give no information regarding the thickness of the envelope in the fresh condition. However, observations made on cells during the process of drying suggest that the maximal thickness in the wet condition could hardly exceed that in the dried condition by more than 50 per cent.

The bearing of the time-thickness curve of lipid extraction by leaching on the probable ultrastructure (mosaic or non-mosaic) will be discussed.

Simultaneous observations on the blood flow of both right and left coronary arteries of anesthetized dogs—effect of drugs. R. G. E. WÉGRIA¹ (by invitation), HIRAM E. ESSEX, J. F. HERRICK and F. C. MANN. Mayo Clinic and Mayo Foundation, Rochester, Minn. (Read by title.)

The experiments were done under chloralose anesthesia and blood flow was measured simultaneously in two coronary arteries by the thermistor-muhr method. The unit was applied to the right coronary artery by an approach through the fourth intercostal space and to the circumflex branch of the left coronary artery through the fifth intercostal space. The chest was closed at the end of each operation and the animal thereafter was able to breathe normally. In a few experiments in which the heart was approached through a midline incision the chest was not closed and artificial respiration was supplied. The mean blood pressure was recorded from the femoral artery and the drugs used were injected into the femoral vein.

Under these conditions the following observations were made: 1. The coronary flow in both the right and the circumflex branch of the left coronary artery measured under these conditions is higher than in trained dogs of the same size. 2. The blood flow in the circumflex branch of the left coronary was at least twice as great as that in the right coronary. 3. Following injections of the drugs used, the changes in flow in the left and right coronary arteries were similar. 4. The following drugs: aminophyllin, nitroglycerine, amyl nitrite, histamine, M. A. P. (muscle-adenosine phosphoric acid), papaverine, caused an increase in the coronary flow, a decrease in blood pressure and an increase in heart rate. The maximal increase in coronary flow was coincident with the maximal fall in blood pressure. 5. Epinephrine caused an increase in coronary flow. The blood pressure and the heart rate were both increased. 6. Pitressin reduced the coronary flow as much as 80 per cent. The blood pressure was increased and the pulse rate was decreased.

¹ Fellow of the Belgian-American Foundation.

A cortin-like action of extracts of human urine. PAUL WEIL¹ (by invitation) and J. S. L. BROWNE. McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

In 1931 Perla and Marmorsten reported a substance in the urine of human adults that raised the resistance of adrenalectomized rats treated with histamine. In 1933 Grollman and Firor reported a method of extracting human urine for cortical hormone. In 1937 Harrop and Thorn reported a method for the extraction from urine of the "salt and water" hormone of the adrenal cortex. In 1938 Anderson, Haymaker and Joseph showed that urine and blood of patients with Cushing's syndrome contained a substance that will maintain life in adrenalectomized rats.

We have used the rapid method of assay devised by Selye and Schenker which depends upon the preservation of life in young rats fasted and exposed to cold. The cortin-like substance is extracted with ethylene dichloride from 24 hour collections of urine. The urine is extracted with 400 cc. (150, 150, 100) ethylene dichloride per 1000 cc. of urine. The ethylene dichloride is distilled off under reduced pressure at an internal temperature not exceeding 50°C. Ten cc. of ethyl alcohol are added to the residue and distilled off to remove the last trace of ethylene dichloride. The residue is then taken up in 25 cc. of distilled water and filtered. The filtrate is placed on ice until used. All extracts are given enterally in a single dose.

It has been found that the urine of certain individuals shows a definite cortin-like action. (The minimum amount that can be shown by the test is 10 units per 24 hours. The highest value was obtained in a case of Cushing's syndrome who excreted 75 units per 24 hours.) These individuals included a case of Cushing's syndrome, four showing hypertension, two of chronic osteomyelitis and two cases after influenza. The urine of some normal individuals at birth and at puberty also showed a cortin-like action. Negative results were obtained in fourteen cases. These individuals included normal adults two of whom were positive when convalescing from influenza, one case of hypertension, and various other diseased states.

*Progestational changes with desoxycorticosterone acetate.*² J. A. WELLS and R. R. GREENE (introduced by L. A. Crandall). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Desoxycorticosterone, synthesized by Steiger and Reichstein, and recently isolated from the adrenal gland by Reichstein, has been reported by Miescher, Fischer and Tschopp to be progestational in doses of less than 10 mg. A study has been undertaken on a series of immature rabbits to determine the progestational activity of desoxycorticosterone acetate.

Immature rabbits were injected for a period of 6 days with a total dose of 150 i. u. of estrone. These animals were then treated for 5 days with crystalline desoxycorticosterone acetate dissolved in peanut oil. Total doses varied from 0.25 to 25.0 mgm. At the end of this period the animals were sacrificed and the uteri prepared for histologic examination. The criteria for the determination of response were those used by McPhail.

It has been found that doses of 20.0-25.0 mgm. will cause a 4 plus

¹ Aided by a grant from the Banting Research Foundation.

² Supported in part by a grant from the Josiah Macy, Jr. Foundation.

response, 15.0 mgm. a 3 plus response, 0.5-10.0 mgm. a 2 plus response, and 0.25 mgm. a questionable 1 plus response. The minimal dose producing a 2 plus response (McPhail rabbit unit) lies between 0.25 and 0.5 mgm.

*Diffusion method for the estimation of acetone in biological fluids.*¹ S. C. WERCH (introduced by M. B. Visscher). Department of Pharmacology, Medical College of the State of South Carolina, Charleston.

This method is based on the recognition of acetone by precipitation of Nessler's solution, discovered by Fearon and Webb, and the use of a diffusion unit, discovered by Conway and Byrne. (W. J. Fearon and D. J. Webb, *Proced. Royal Dublin Society*, March, 1936. E. J. Conway and A. Byrne, *Biochem. J.* **27**: 419, 1933.)

In the estimation of acetone one considers the time necessary to cause precipitation of the Nessler's solution, and the temperature at which the reaction takes place. When the unknown acetone solution is introduced in the outer chamber of the Conway unit, a stop-watch is started, and the unit covered with a transparent, glass plate. Precipitation takes place from the periphery of the inner chamber of the unit, into which the Nessler's solution is introduced, in the form of threads, like spokes of a wheel, or as a cloud. When the spokes or cloud extend inward one millimeter the stop-watch is stopped. The quantities used are as follows: 2 cc. of Nessler's solution; 3 cc. of unknown; and 1 cc. of dilute acid, distributed evenly around the outer chamber to fix any ammonia which may be present.

At 16°, a 1 per cent solution of acetone causes precipitation of the Nessler's solution in 6 seconds, a 0.01 per cent solution in 19 seconds, a 0.001 per cent solution in 60 seconds. As regards time and dilution the parabolic formula $t^2/D = K$ is followed. Temperature changes are associated with regular changes in the time factor. Including temperature the following general formula may be used:

$$D = \frac{179t^2}{(24 - \theta)^2}$$

D = dilution, t = time, θ = temperature.

These formulas may be incorporated in a chart.

Specimens of blood and urine from patients in acidosis have been tried and found to give fair results.

The effect of ageing on gonadotropic extracts of pregnancy urine. M. J. WERNER (by invitation), M. B. LONG (by invitation) and J. S. L. BROWNE. McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

Assays to determine changes due to ageing in the potency of pregnancy urine extract (A. P. L. in Solution) were carried out on immature female rats of a hooded strain 22-23 days of age and weighing 45-55 grams. The extracts were assayed two weeks after preparation and at periods from five months to two years after the preparation of the extracts.

In some extracts assayed within two weeks of preparation the dose necessary to produce corpora lutea was ten to twenty times, in others two to three times that necessary to produce positive vaginal smears.

¹ This work was begun at Trinity College, Dublin.

On ageing both types of extracts at ice-box temperature, these ratios did not change appreciably over the period indicated.

It was at first thought that aging caused a loss of corpus luteum producing ability since the older extracts all showed a low potency in this regard. This proved to be probably incorrect, since some extracts assayed two weeks after preparation showed the same low corpus luteum producing potency relative to their oestrus producing effect as did the old extracts, and a fresh extract showing high activity, maintained the same activity when assayed one year later.

Both types of extract were able to cause thecal luteinization in hypophysectomized rats. In the low potency extracts the ability to produce corpora lutea was greatly augmented by the addition of small quantities of extract of mare's serum (Antex). In one instance amounts of A. P. L. and Antex which alone produced only 50 per cent positive oestrus were able when mixed in vitro to produce corpora lutea in 80 per cent of the rats used.

Hypophysis and experimental diabetes insipidus. H. L. WHITE and PETER HEINBECKER. Departments of Physiology and Surgery, Washington University, Saint Louis, Mo.

In 9 dogs hypothalamic lesions designed to destroy the pars nervosa were followed in 5 to 18 weeks by attempted complete hypophysectomy. Serial sections of brain and skull showed isolated microscopic fragments of anterior lobe remaining in four, from 5 to 10 per cent of the original in four and 20 per cent in one. In no case did such hypophysectomy diminish within a few weeks the preëxisting polyuria. After several weeks the polyuria began to diminish, but, with rare exceptions, this has also been our experience with polyurias where the anterior lobe was left essentially intact. It is possible but not certain that the polyuria remains at or near its maximum longer when some anterior lobe is present. Interpretation is complicated by the fact that it has not been possible to get a series with varying degrees of anterior lobe removal and uniformly complete pars nervosa destruction. Completeness of removal of infundibular process and stalk being accomplished, the appearance of the supraoptic and filiform nuclei and of the median eminence is taken as further criteria of completeness of destruction of pars nervosa.

In 5 other dogs, attempted complete hypophysectomy (gland plus stalk plus median eminence) in one stage was proved by serial section to be successful in all except for varying degrees of destruction of the median eminence. Polyuria resulted in every case, but in no case was it permanently maximal. However, in no case was complete destruction of pars nervosa attained; it is not definitely established in this series that failure of maximum polyuria is due to absence of anterior lobe. The polyuria of such complete hypophysectomy shows no normal interphase.

There are 2 surviving dogs with high and permanent polyuria in which complete removal of anterior lobe was attempted. Marked testicular atrophy has occurred but demonstration of complete absence of anterior lobe will depend upon serial section findings.

Testicular atrophy is not an adequate criterion of complete anterior lobectomy in the dog; it occurs with up to about 15 per cent of the lobe remaining.

The effect of gastric juice, administered to the pregnant mother, on the erythrocytes of newborn rats. H. S. WIGODSKY and T. A. NOBLE (introduced by F. T. Jung). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The parenteral administration of concentrated gastric juice or the oral administration of unaltered gastric juice to the pregnant white rat failed to influence significantly the number, size, and hemoglobin content of the erythrocytes of the newborn pups. Daily administrations of 2 cc. of human gastric juice concentrated ten times *in vacuo* (11 litters, 71 pups) or 4 cc. of the unaltered gastric juice (15 litters, 101 pups) were made 4 to 10 days before delivery (controls: 20 litters, 144 pups).

Central and chemo-reflex influence of potassium excess on circulation and respiration. CLAUDE V. WINDER. Department of Physiology, University of Michigan, Ann Arbor. (Read by title.)

At the 1938 meetings these observations on dogs under morphine and chloralose or nembutal were presented but inadequately abstracted. They are essentially similar to results on chloralosed cats submitted for publication in May, 1938 by von Euler, and should therefore be on record.

Quick intravenous injections of 2.5–15.0 cc. of isotonic KCl elicited transient hyperpnea, sometimes with secondary depression and tertiary augmentation. The blood-pressure changes of Mathison were present. With Hering's and vagus nerves cut breathing was uninfluenced or depressed.

With the carotid bifurcation *completely* isolated arterially excepting the lingual artery, quick intracarotid injections of 0.01–1.0 cc. elicited the hyperpnea and cardiovascular changes typical of carotid body excitation. The greater responses occurred with the smaller doses and were inferior to the maximal, e.g. with cyanide. Rapid repetition or largeness (1.0 cc.) of injection diminished the excitation, replacing it by paralysis indicated by inexcitability by cyanide, the subnormal breathing often seen on cutting Hering's nerve, and frequently more or less maintained drop in blood pressure. Cutting Hering's nerve eliminated these effects.

With the bifurcation denervated and major branches ligated except the internal carotid and occipital arteries, intracarotid injections of 0.1–1.0 cc. elicited just discernible to severe hypopnea or apnea concurrent with vascular and (vagi intact) cardiac depression like that of vascular pressoreceptor stimulation. With the larger doses, convulsive twitches of head and cervical muscles were often seen; the apnea was often of a restless type—fixation in mid-inspiratory or supranormal expiratory position, occasionally followed by hyperpnea more than equivalent to the apnea, and infrequently initiated by a violent, clonic inspiratory spasm. The vascular depression was often followed by prolonged hypertension with the larger doses. All this suggests excitation rather than depression—i.e. excitatory effect of the potassium at central structures, e.g., in the nucleus fasciculus solitarius, predominantly inhibitory to vasomotor action, excitatory to cardio-vagal fibers, incoordinately excitatory (and inhibitory?) to both inspiratory and expiratory mechanisms.

Large doses trapped in the sinus proper caused excitation then paralysis of pressoreceptors.

Any small arterial leak from the bifurcation usually provided admixture of reflex and central effects with doses over 0.25 cc.

Effect of pitressin injections upon the serum electrolytes and water exchange of cats with diabetes insipidus and adrenal insufficiency. CHARLES A. WINTER (by invitation), W. R. INGRAM and E. G. GROSS. Departments of Physiology, Anatomy and Pharmacology, State University of Iowa, Iowa City.

Cats with diabetes insipidus produced by interruption of the supra-opticohypophyseal tract have normal concentrations of serum sodium, chloride and potassium. Subcutaneous injection of 3 pressor units of pitressin three times daily has no effect on the concentration of serum electrolytes either in normal or in d.i. cats. Such injections control the polyuria of d.i. animals, but have little effect on the water exchange of normal cats. D.i. cats survive bilateral adrenalectomy for only 2.9 days on the average, compared with 7.5 days for non-polyuric animals. Pitressin injections do not affect the survival time of non-polyuric adrenalectomized animals, and extend the survival time of d.i. adrenalectomized cats to about the same as the non-polyuric. Serum sodium and chloride fall during adrenal insufficiency in non-polyuric cats with or without pitressin, and in d.i. cats with pitressin, but not in d.i. animals without pitressin; serum potassium is elevated in all groups following adrenal removal. The average daily water retention (total intake - urine volume) in 4 non-polyuric controls was 39 cc. lower after adrenalectomy than before. Similar water balance figures were obtained following adrenalectomy for non-polyuric cats on pitressin and for d.i. cats on pitressin, but in 10 polyuric animals the average daily water retention was 96 cc. lower during adrenal insufficiency than before. The survival time of the latter is much shorter, however, and the total water lost before death is slightly less than in any of the others. When d.i. cats are given pitressin, their total water loss in adrenal insufficiency is about 28 per cent greater than similar animals not treated, yet they survive more than twice as long on the average; therefore, total water loss by way of the kidney does not account for the early deaths of the untreated polyuric-adrenalectomized animals. In summary, d.i. cats on pitressin after adrenalectomy behave like the control animals with respect to survival time, serum electrolyte changes, and water balance, while without pitressin they differ from the controls in these respects. This suggests the possibility that the posterior lobe may participate in both electrolyte and water exchange.

Studies in pain sensation. II. The quantitative analysis of the action of analgesics. H. G. WOLFF, J. D. HARDY (by invitation) and H. GOODELL (by invitation). Russell Sage Institute of Pathology in affiliation with the New York Hospital, and Department of Medicine, Cornell University Medical College, New York City. (Read by title.)

For the method of measuring pain thresholds see *Studies in pain sensation, I*. The threshold was established to ± 2 per cent. After giving the drug the change in threshold was followed until all threshold raising action had ceased. The analgesic action was considered from two viewpoints: 1), the height of the threshold raising effect, and 2, averaged action over the effective period.

Morphine sulphate: Effects 1 and 2 increased approximately proportional to the amount administered up to 15 mgm. (.23 mgm./kgm. body weight) (hypodermic). Doubling this amount increased these actions Ca 15 per cent and 17 per cent respectively. That these effects of morphine were non-peripheral was demonstrated by testing its action on a ligated limb. Pronounced side effects were experienced.

Codeine phosphate: Effect 1 was proportional to the amount up to 60 mgm. (0.92 mgm./kgm. body weight) (hypodermic). A "ceiling" in effect 1 was reached at 55 per cent over the control. Effect 2 increased in proportion to the amount up to 120 mgm. Pronounced side effects with larger doses were experienced.

Acetylsalicylic acid: Effect 1 was the same for amounts from 0.3 gram (4.6 mgm./kgm. body weight) to 1.8 gram (oral). The total effect increased with amount up to about 0.6 gram, beyond which increasing the dose caused relatively little increase in the total effect. The "ceiling" of effect 1 was about 35 per cent over the control level. Few side effects were experienced.

Other agents: Effect 1 of acetanilid and acetpheneditin (oral) was shown to be slightly lower than that of acetylsalicylic acid; the side effects were more evident. A combination of acetanilid, acetpheneditin, acetylsalicylic acid, each 0.3 gram, had no greater effects than an equivalent weight (0.9 grams) of acetylsalicylic acid. A combination of acetylsalicylic acid, 0.3 gram, and codeine, 60 mgm., had no greater effects than that of 60 mgm. of codeine. The barbiturates had relatively slight analgesic action (Ca 20 per cent) and in combination with acetanilid or acetylsalicylic acid no synergistic action was noted. Alcohol raised the threshold of pain rapidly to Ca 35-40 per cent. The action was of short duration.

Psychologic, hypnotic, and other side effects experienced with the above agents were in no way related to the analgesic action, beginning and ending independently.

Potassium exchanges between mammalian muscle and blood in relation to activity. E. H. WOOD, D. A. COLLINS, and G. K. MOE (introduced by M. B. Visscher). Department of Physiology, University of Minnesota, and Temple University, Philadelphia, Pa.

The time relations of potassium and water exchanges during rest, stimulation, and recovery have been studied by analysing the arterial and venous blood and plasma of an isolated dog gastrocnemius perfused by means of a heart-lung preparation. Average results of the whole blood analyses on experiments run on eight preparations using periods of from 100 to 250 seconds of indirect stimulation are given in the table.

Potassium and water exchanges for all periods were calculated on the basis of dry weight venous flows, assuming that the dry weight of the blood changed only slightly during passage through the muscle.

The more important results are: 1. A relatively rapid loss of potassium (about 2 mgm. per 100 gm. muscle) occurs during stimulation. In the majority of experiments the rate of potassium exchange was zero or reversed within the first 30 seconds of recovery. 2. The muscle gained about 1 gram of water per 100 grams muscle during stimulation. This gain continued at a progressively slower rate for periods of recovery up to

| | ARTE- RIAL | VENOUS | | | | |
|----------------------------|---------------|--------|------------------|----------|-------|-------|
| | | Rest | Stimu- lation | Recovery | | |
| | | | | 30' | 6' | 11' |
| K mgm./100 gm. dry weight | 99.4 | 101.6 | 116.3 | 96.9 | 98.8 | 98.8 |
| Per cent H ₂ O | 81.60 | 81.64 | 80.99 | 81.25 | 81.51 | 81.54 |
| Cl gm./100 gm. dry weight | 1.66 | 1.66 | 1.63 | | 1.67 | 1.66 |
| Per cent cell volume | 40.8 | 41.7 | 42.9 | | 43.0 | 42.7 |
| Cc./min. venous blood flow | | 10.0 | 30.0 | 35.0 | 21.0 | 17.0 |

10 minutes. 3. Direct stimulation of a normal muscle, a curarized muscle, and a muscle the motor nerve to which had been transected 13 days previously resulted in potassium exchanges similar to those shown above.

The proprioceptive drive of the respiratory act. JOSEPH J. WORZNAK (by invitation) and ROBERT GESELL. Department of Physiology, University of Michigan, Ann Arbor.

Properly timed inflation of the lungs during the phase of inspiration was found to increase the frequency of unicellular firing and the recruitment of active muscle fibers in inspiratory muscles (diaphragm, internal intercostal and external intercostal). These findings are in agreement with the fact that normal vagal action speeds the act of inspiration, that lung inflation increases the effect, that vagal block decreases it, and that a combination of complete vagal inhibition of breathing plus a minor acceleration from cutaneous sensory stimulation produces a paradoxical acceleration of breathing. They support the thesis that the vagal proprioceptive signals are inspiratory excitatory, that progressive inflation of the lungs produces a progressive bolstering of the inspiratory act and not an inhibition as maintained by Adrian.

Pulmonary inflation during the expiratory pause commonly augments the strength of the abdominal expiratory muscles. This effect is abolished by vagotomy and indicates that the vagal proprioceptive signals are expiratory excitatory as well as inspiratory excitatory.

The inhibition of breathing produced by pulmonary inflation during the expiratory pause is not an inspiratory inhibitory phenomenon but a central motor reciprocal inhibition accessory to a reflex excitation of the expiratory muscles.

Mechanical obstruction to inspiration increases enormously the intensity of inspiratory contraction. This is due to a purposively graded stretch reflex elicited through the Golgi endings. Golgi proprioceptive signals like vagal signals bolster the inspiratory act to meet the progressively increasing resistance of inspiration.

Inflation of the lungs after double vagotomy has in some experiments reduced the strength of abdominal expiratory contraction indicating that muscle spindles exert an inhibitory action. Since muscle spindles are relieved of strain in contracting muscle, it is tentatively suggested that a diminishing number of signals serve to strengthen a contraction of increasing intensity.

It is proposed that proprioceptive augmentation of contraction is an important principle in motor integration.

Maximum blood flow in the human lower extremity. GEORGE W. WRIGHT and KENTON PHELPS (introduced by C. J. Wiggers). Departments of Physiology and Surgery, Western Reserve University, School of Medicine, Cleveland, O.

Using an optical modification of the plethysmograph based on the method first described by Hewlett and Van Zwailenberg we have compared five methods to each of which has been attributed the capability of producing *maximal blood flow* in the extremities. We define *maximal blood flow* as the largest quantity of blood that can be caused to flow through unit volume of tissue in unit time with a constant resting mean blood pressure. The measurements have been made on the right leg and foot, the room temperature being constantly between 27 and 28 degrees centigrade.

Diathermy applied to the sacral plexus (de Takats) at times produced an increase, at other times a decrease in blood flow as compared to the resting level. A further increase was always produced by immersing the plethysmograph in water at 45°C.

Immersion of the upper extremity in water at 45°C. for 30 minutes (Gibbons and Landis) always produced an increase in blood flow as compared to the resting level but a further increase was always produced by local heating.

Spinal anesthesia (Morton and Scott) resulting in complete motor and sensory block from D-12 down failed to produce any significant increase in blood flow as compared to the resting flow. Immersion of the upper extremity or the measured part in water at 45°C. produced a marked increase in blood flow in the presence of the anesthesia.

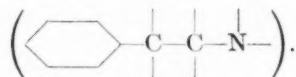
Sciatic nerve block (White) produced constantly a marked increase in blood flow as compared to the resting level. Immersion of the upper extremity or the measured part in water at 45°C. failed to augment this flow significantly.

Heating the part being measured in water at 45°C. for 30 minutes produced a flow of blood of essentially the same value as produced by sciatic nerve block and immersion of the upper extremity did not augment this flow.

Conclusion: Local heat and sciatic nerve block produce maximal blood flow.

Relation of various groups of the adrenalin molecule to its smooth muscle inhibiting function. W. B. YOUNG and K. AUMANN (introduced by W. J. Meek). University of Oregon Medical School, Portland. (Read by title.)

Equivalent intestine-inhibiting injection rates have been determined for six "adrenalin-like" compounds which differ from adrenalin by lacking one or two of the groups which distinguish adrenalin from the fundamental sympathomimetic nucleus designated by Barger and Dale



Records of intestinal motility were obtained by the balloon-mercury-manometer method from six dogs having a total of ten Thiry or Thiry-

Vella fistulae. Four of the fistulae were denervated. Apparatus was used which permits a constant injection at any desired rate.

The inhibitory effects of adrenalin on intestinal motility may be duplicated by any one of the compounds when it is injected at the proper rate. After denervation the intestine becomes hypersensitive to each of the compounds.

On the basis of quantitative studies the compounds are listed below in the order of their potency as inhibitors of the intestine, the most potent being placed first. The name of the compound is followed by a phrase indicating how the compound differs from adrenalin. The figures in parenthesis indicate the extremes for the number of times the injection rate must be increased in order to duplicate the effects of a given adrenalin injection rate. 1. l-Adrenalin, (1). 2. dl-Arterenol, methyl group on the nitrogen atom lacking, ($1\frac{1}{2}$ -4). 3. Cobefrin, methyl group on nitrogen lacking, and having an additional carbon atom in the side-chain, ($2\frac{1}{2}$ -10). 4. Epinine, lacking the secondary alcohol group, (10-25). 5. Kephrene, ketone instead of secondary alcohol group, (25-100). 6. l-Neosynephrin, lacking the para-hydroxy group, (25-100). 7. dl-Synephrin, lacking the meta-hydroxy group, (500-2500).

The order of potency for intestinal inhibition differs from the order of potency for the same compounds in causing contraction of the nictitating membrane (Bacq, 1936 and 1938). This may possibly be related to a difference in the "receptive mechanisms" of the two types of smooth muscle.

Nucleoproteins from streptococcus pyogenes: some chemical and serological properties and changes in both caused by certain enzymes. CHARLES A. ZITTLE (introduced by Stuart Mudd). Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia.

A nucleoprotein (M) has been extracted from whole organisms with N/20 HCl. Serologically M reacts type specifically like the Lancefield "M". M has been compared with a nucleoprotein (NPA) obtained by sonic disintegration of the organisms and extraction at pH 6-7. NPA has been described previously in reports from this department.

M gave a negative Millons and Hopkins-Cole reaction; color tests were positive for ribose nucleic acid. NPA gave strongly positive Millons and Hopkins-Cole reaction and positive color tests for both ribose and desoxy-ribose nucleic acid. NPA was relatively unstable in N/20 HCl. The presence of ribose nucleic acid in M and NPA was confirmed by the specific action of ribonucleodepolymerase.

Destruction of the type specificity of M by trypsin was confirmed and a simultaneous loss of precipitability with acid was found. This was rapid; 0.05 mg. of trypsin per cc. of a 0.2 per cent solution destroyed acid precipitability within one minute and serological tests after three minutes showed the specific precipitation also was destroyed. Precipitation of the tryptic digests of M with alcohol gave a high phosphorus, biuret negative component. A biuret positive, low phosphorus, non-diffusible component was obtained from the alcoholic solution.

Trypsin destroyed the precipitation of NPA with NPA antisera but not with whole organism antisera. NPA could not be precipitated at pH 3-4 but a precipitate was obtained at pH 1. Commercial trypsin was found to destroy precipitation at this lower pH also. This was probably caused by the ribonucleodepolymerase in commercial trypsin.

THE COAGULANT ACTION OF CRYSTALLINE TRYPSIN, CEPHALIN AND LUNG EXTRACTS

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According to current opinion, the coagulation of blood fibrinogen is effected by thrombin produced from plasma prothrombin via an "intermediary complex" containing calcium and cephalin (4, 5). The function of the last named has been termed *thromboplastic* (10).

In greatly extending Northrop¹ and Kunitz's (16) observation of the clotting power of their crystalline trypsin, Eagle (2) apparently overlooked the fact, subsequently re-emphasized by the Rockefeller workers (13), that the addition of a trace (0.002*M*) of calcium was essential for the trypsin clotting of diluted (1:10) MgSO_4 plasma.

In our experiments it has been found that crystalline trypsin can indeed clot ordinary citrated plasma and activate prepared prothrombin without added calcium or cephalin. Nevertheless, the enzyme is much more potent in the presence of ionized calcium. Furthermore, excess of citrate can inhibit its clotting activity. These significant facts are easily verified (tables 1 and 2).

Added cephalin (in contrast to Eagle's "tissue extract") is decidedly of advantage also, though not as strikingly when the enzyme is used alone as for the development of optimal potency in the presence of calcium salts (tables 3, 4).

Northrop (17) commented on the remarkable thermostability of crystalline trypsin. This is shown to be true of its coagulant function in the boiling experiments (table 3). As compared with the small but definite impairment in the enzyme used alone, or with cephalin only, there was no significant loss of activity in the preparations subsequently recalcified, especially in the presence of cephalin.

In the thrombin formation data of table 4 is seen the marked thromboplastic action (5) of cephalin on a prothrombin solution which is very poorly activated by calcium alone. There is no evident thrombin formation with too much trypsin because of the proteolytic destruction of

¹ We are greatly indebted to Doctor Northrop for our supply of enzyme.

thrombin by the enzyme (2). Calcium, alone and with cephalin, does not prevent this. A smaller concentration is effective (v. supra) and cephalin

TABLE 1
*Effects of calcium and of excess citrate on clotting of citrated dog plasma by crystalline trypsin**

| | CITRATE PLASMA | CaCl ₂ (N/10) | SOD. CITRATE, ISOTONIC (3.8%) | CRYSTALLINE TRYPSIN | CLOTTING-TIME (38°C.) |
|----|----------------|-----------------------------|----------------------------------|------------------------|--------------------------|
| | cc. | cc. | cc. | mgm. | |
| 0 | 1 | 0.25 | | | 95" |
| 1 | 1 | | | 1.0 | 40" |
| 2 | 1 | | | 0.5 | 55" |
| 3 | 1 | | | 0.25 | 90" |
| 4 | 1 | | | 0.05 | ∞ |
| 5 | 1 | | | 0.025 | ∞ |
| 6 | 1 | 0.25 | | 0.5 | 16" |
| 7 | 1 | 0.25 | | 0.05 | 30" |
| 8 | 1 | 0.25 | | 0.025 | 93" |
| 9 | 1 | | 0.05 | 1.0 | 63" |
| 10 | 1 | | 0.5 | 1.0 | 83' |
| 11 | 1 | | 0.05 | 0.5 | 85" |
| 12 | 1 | | 0.5 | 0.5 | ∞ |

* Dilution factor controlled by using constant volume (0.5 cc.) of clotting agent.

TABLE 2
Effects of calcium and of citrate on the activation of prothrombin by crystalline trypsin

Thrombic mixtures (T) = 4.0 cc. prothrombin solution + 1.0 cc. activating agents (suitably diluted). Calcium and citrate expressed in milli-equivalents *per* 5.0 cc. of thrombic mixture, trypsin in milligrams. Thrombin formation at 15°C. Clotting-times (seconds) for 0.5 cc. T + 1.0 cc. fibrinogen solution* (prothrombin-free), at 38°C.

| T | ACTIVATING AGENTS IN THROMBIC MIXTURE | THROMBIN INCUBATION PERIOD (MINUTES) AT 15°C. | | | | | | | |
|---|---------------------------------------|---|-------|-------|------|-------|------|-------|------|
| | | 10' | 20' | 30' | 45' | 60' | 75' | 90' | 120' |
| 1 | Calcium (0.05) | ∞ | 3300" | 1060" | 390" | 210" | 110" | | |
| 2 | Trypsin (0.1) | ∞ | ∞ | ∞ | ∞ | 240" | 45" | 45" | |
| 3 | Trypsin (0.02) | ∞ | ∞ | ∞ | ∞ | 2400" | | 1200" | 840" |
| 4 | Trypsin (0.1) + Ca (0.05) | † | 40" | 32" | | 26" | | | |
| 5 | Trypsin (0.02) + Ca (0.05) | 570" | 120" | 60" | | 47" | | | |
| 6 | Trypsin (0.1) + Citr. (0.1) | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| 7 | Trypsin (1.0) + Citr. (0.1) | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |

* Dehydrated fibrinogen (7) dissolved in citrated (0.38 per cent) saline (0.9 per cent NaCl) and filtered clear. No clot in 24 hours (at 38°C.) with Ca + cephalin.

† Thrombinolysis.

plus calcium gives better potentiation of the enzyme action than does calcium alone. Experiment 8 again shows the thermostability of the boiled enzyme.

Relationship of the clotting function of trypsin to its ability to digest proteins (cf. 2, 12, 18). Thrombins activated without trypsin practically never induce lysis in the subsequent clots, in our experience, whereas clots obtained with trypsin-thrombins show syneresis (clot-retraction) and fibrinolysis within a few minutes. Some 1-2 mgm. of crystalline trypsin, acting alone, are optimal for the clotting of 1 cc of citrated dog plasma.

TABLE 3

Effects of added cephalin and calcium on the clotting time of trypsinized citrate plasma (dog). Thermostability of trypsin

| | TRYPSIN SOLUTION (0.5 cc. = 0.5 mgm.) | DISTILLED WATER | CEPHALIN 1:1000 | CaCl ₂ N/10 | CITRATE PLASMA | CLOTTING-TIMES (SECONDS) AT 38°C. | | |
|---|---|--------------------|--------------------|---------------------------|-------------------|-----------------------------------|--------------------------------|--------------------------------|
| | | | | | | A. Un-boiled trypsin | B. Trypsin boiled 1 min. | C. Trypsin boiled 5 min. |
| | | cc. | cc. | cc. | cc. | | | |
| 1 | | 0.75 | | 0.25 | 1.0 | 365" | | |
| 2 | | 0.5 | 0.25 | 0.25 | 1.0 | 78" | | |
| 3 | 0.5 | 0.5 | | | 1.0 | 150" | 200" | 270" |
| 4 | 0.5 | 0.25 | 0.25 | | 1.0 | 107" | | 225" |
| 5 | 0.5 | 0.25 | | 0.25 | 1.0 | 40" | 45" | 52" |
| 6 | 0.5 | | 0.25 | 0.25 | 1.0 | 22" | 22" | 22" |

TABLE 4

Effects of calcium and added cephalin on the activation of prothrombin by trypsin

Similar experimental materials and conditions to foregoing (table 2). *Cephalin* expressed in milligrams per 5.0 cc. of thrombic mixture.

| T | ACTIVATING AGENTS IN THROMBIC MIXTURE | THROMBIC INCUBATION PERIOD (MINUTES), AT 15°C. | | | | | | | | | |
|---|---|--|------|------|------|------|-----|------|------|------|------|
| | | 2' | 5' | 10' | 20' | 30' | 45' | 60' | 90' | 120' | 180' |
| 1 | Calcium (0.025) | | 203' | 167' | 55' | 28' | 19' | 13' | 190* | 67' | 47' |
| 2 | Ca (0.025) + cephalin (0.25) | | 47" | 35" | 25" | 22" | 19" | 18" | 18" | 18" | 18" |
| 3 | Trypsin (1.0 mgm.)* | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ |
| 4 | Trypsin (0.2 mgm.)* | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | ∞ | | | |
| 5 | Trypsin (0.05 mgm.) | ∞ | ∞ | ∞ | 120* | 40" | | 25" | | | |
| 6 | Trypsin (0.05) + Ca (0.025) + cephalin (0.25) | 40" | 27" | 20" | 20" | 20" | | 20" | | | |
| 7 | Trypsin (0.05) + Ca (0.025) | | 180" | 145" | 25" | 20" | | 20" | | | |
| 8 | Trypsin (0.05) (boiled) + calcium (0.025) | | 430" | 130" | 120" | 120" | | 120" | | | |

* Calcium, alone and with cephalin, failed to prevent thrombinolysis.

Quantities of the order of one-fiftieth to one-hundredth of this are inactive alone but, when potentiated by calcium, yield quick, solid clots that fail to lyse in 1 to 2 days at 38°C. It is concluded that syneresis, fibrinolysis and thrombinolysis are incidental to the proteolytic action of the enzyme. Its clotting action is exhibited prior to the lytic phenomena and, if calcium be present, with very much smaller concentrations of enzyme.

Nature of the clotting rôle of crystalline trypsin. Eagle (2) dialysed trypsin to lessen the remote possibility of a significant calcium content and pointed out the high dilution factor which, alone, would remove this possibility from serious consideration.

Analysis of 50 mgm. of Northrop and Kunitz's trypsin failed to reveal any phospholipid. Moreover, after the exhaustive alcohol-ether extraction, the enzyme residue readily redissolved in water to give solutions which appeared, in comparison with fresh solutions of equal enzyme content, to have suffered no diminution of thromboplastic and fibrinolytic potency.

The most conclusive argument against the possibility of trypsin being a thrombin, or a precursor of thrombin, is the complete inability to clot a fibrinogen, meticulously free from prothrombin (6), even when calcium and cephalin are also added. *Prothrombin*, in our experience, is never activated "spontaneously." Contradictory experiments in the literature (1, 14) must be subject to technical criticism. A logical explanation is that they fail to control the new factor which, like trypsin, may be thromboplastic even under conditions of great depression of calcium ionization. Thrombin, sufficiently "ripened" (4), clots fibrinogen or plasma with very little delay even in the presence of considerable excess of citrate. Trypsin, treated with like quantities of calcium and cephalin, fails to clot the citrated reagents or, in plasma experiments with small excess of citrate, is greatly delayed in its action. The resistance of prothrombin to ageing and, partially, to boiling, is not a valid criterion of difference in view of the thermostability of pure trypsin, but it may be true that, in the presence of other proteins, the enzyme is less stable.

Mode of action of trypsin, with particular reference to the "availability" of cephalin. Analyses reveal some 8 to 30 mgm. per cent of phospholipid, varying with dilution, in our prothrombin solutions. Approximately 40 to 60 per cent is choline-free, i.e., "cephalin." This amount of P-lipid, if supplied in the form of added cephalin solution would be powerfully thromboplastic. Yet calcium alone is hardly able to activate the prothrombin. In fact, prothrombins have been prepared (3) which give no thrombin on simple recalcification but require cephalin or other thromboplastic agent. It is concluded that the phospholipids bound up with the proteins of the preparation are inactive in thrombin formation. Can it not be the function of the proteolytic enzyme to set them free?

No evidence of "free" phospholipid could, with certainty, be obtained in plasma, prothrombin solutions or aqueous tissue extracts by such methods as *a*, simple extraction with fat solvents, e.g., ether, petroleum ether, benzene; *b*, ultrafiltration through collodion and cellophane membranes; *c*, after dehydration, whether in vacuo at the temperature of "dry ice" or in air at room temperatures, and with plaster of Paris; *d*, after

heating to the point of complete precipitation of the proteins.² It appears probable that these negative results are highly significant in proving that all the phospholipid of these agents exists only in firm combination with proteins. Digestion of prothrombin solutions, in one or two instances, using 1 mgm. of trypsin per 100 cc., yielded 4 to 5 mgm. per cent of phospholipid which could be recovered by simple ether extraction after drying in casing sacs.

It is concluded that proteolytic enzymes split off cephalin from its inert protein combinations and make it "available" for the activation of prothrombin. We should like to introduce the term *thromboplastic enzymes* for all proteases which assist blood clotting in this way. Briefly their action consists in the mobilization of cephalin and calcium at the colloidal surface of the protein (prothrombin) substrate, where the close juxtaposition of the three components permits of the formation of thrombin according to the plan which we have outlined on a previous occasion (5), namely, via an "intermediary" prothrombin-calcium-cephalin complex or compound.

This conclusion necessitates a revision of our concepts as to the exact physical state of calcium during thrombin formation. Orientation at a colloidal surface may take the place of diffuse ionization. Amongst other things, this may explain the occasional occurrence of clotting in citrated or oxalated blood samples.

Once we can bring the enzyme action into line with the classical processes of thrombin formation, an entirely new viewpoint is attained and we can now begin to look for thromboplastic enzymes as part of the normal physiological clotting mechanisms. Of the ubiquity of the proteases there can be no doubt. Schmitz (19) has recently isolated trypsin from blood plasma, and Izumi et al. (11) have noted tryptic activity in lung extracts.

The analytical data of table 5 are of interest in relation to the recognized possibility that tissue *thromboplastins* may serve as sources of phospholipid. All analyzed extracts were solutions from *lyophilized* (cf. 7) lung material, excepting 3, which was made from frozen tissue (17 days old).

Table 6 includes a qualitative comparison of the thromboplastic activity of an aqueous lung extract, boiled and unboiled, with its extracted phospholipids and an inferior cephalin preparation. Mills (15) attributed the residual activity after boiling to the thermostable phospholipid component and also noted that added cephalin improved the thromboplastic potency. In view of the thermostability of trypsin (v. supra), the cited conclusion is open to criticism. We have obtained very variable results on boiling tissue extracts; sometimes even an enhancement of potency (cf. 3). A fresh crude tissue extract (cf. 21) is much more thromboplastic than an

² This research, which is being continued by one of us (B. N. E.) with the aid of a Sigma Xi grant in collaboration with the Department of Pediatrics at the University of Michigan, will be reported in a subsequent communication.

equivalent solution of its isolated P-lipids. While it is clear that the tissue phospholipids, when artificially isolated, have a thromboplastic action which may very well be due to the cephalin content, there is the above-mentioned inability to demonstrate such "free" cephalin experi-

TABLE 5

Analyses of lung extracts (Van Slyke N and P methods)

All quantities expressed in milligrams per 100 cc. of filtered solution

| | DRY WEIGHT* | ASH* | TOTAL N | N.P.N. | PRO-TEIN (= DIFF. X 6.25) | TOTAL P-LIPID | CHO-LINE P-LIPID | "CEPH-ALIN" P-LIPID (BY DIFFERENCE) |
|------------------------------|-------------|------|---------|--------|------------------------------|---------------|------------------|--|
| 1. Unperfused (aqueous)..... | 430 | | 52.55 | 19.0 | 209.7 | 14.3 | 2.60 | 11.7 |
| 2. Unperfused (saline)..... | 425 | | 60.5 | | | 18.4 | 2.57 | 15.83 |
| 3. Perfused (aqueous)..... | 235 | 25 | 19.1 | 4.3 | 95.7 | | | |
| 4. Perfused (aqueous)..... | 235.5 | | 21.2 | 4.36 | 105.3 | 6.0 | 0.84 | 5.16 |
| 5. Perfused (saline)..... | 210 | | 20.5 | | | 7.15 | 0.60 | 6.55 |

* These weights corrected for 0.9 per cent NaCl in case of saline extracts and for 5 mgm. per cent NaHCO₃ in both saline and aqueous extracts.

TABLE 6

*Thromboplastic activity of lung extracts and isolated lung phospholipids**

Prothrombin activation curves by technique described in table 2. Phospholipids expressed in milligrams per 5 cc. of thrombic mixture; lung extracts in cubic centimeters.

| T | ACTIVATING AGENTS IN THROMBIC MIXTURE | THROMBIN INCUBATION PERIOD (MINUTES) AT 15°C. | | | | |
|---|--|--|------|-------|-------|------|
| | | 5' | 10' | 20' | 30' | 60' |
| 1 | Calcium (0.05 m.-eq.) | ∞ | ∞ | 3300" | 1060" | 210" |
| 2 | Ca (0.05) + lung extract (0.5 cc.) | 140" | 50" | 25" | 20" | 16" |
| 3 | Ca (0.05) + <i>boiled</i> lung extract (0.5 cc.) | hours | 720" | 255" | 120" | 37" |
| 4 | Ca (0.05) + lung P-lipids* (0.14 mgm.) | | 15" | 14" | 14" | 14" |
| 5 | Ca (0.05) + cephalin (0.5 mgm.)† | | 16" | 15" | 15" | 15" |

* Perfused lung extract exhausted with alcohol-ether (3:1), acetone-insoluble lipids recovered in ether, dried, and taken up in aqueous solution.

† The cephalin preparations used in all the cited experiments are from an old stock suspension kept under absolute alcohol. It has hydrolysed to some extent and lost (we believe) some $\frac{1}{3}$ of its original thromboplastic potency.

mentally. The question of its availability, therefore, appears to require an hitherto unidentified factor in the tissue extract. The experimental analogy to weak trypsin (? plus cephalin) is *exact*. Both enzyme and tissue preparation require calcium in order to exert an action which is typically thromboplastic and somewhat enhanced by "free" cephalin.

Both are inactive in the presence of citrate or oxalate (cf. 15) but resist heparin (9). Pending isolation, the factor thus defined is provisionally identified with a weak proteolytic enzyme. Fibrinolysis of the clots typically produced in the presence of tissue extracts and thrombinolysis of the blood prothrombin in fresh *citrated* (occasionally, saline (21)) extracts of unperfused³ lung are two minor but perhaps significant pieces of evidence supporting the protease theory.

The identification in the thrombic system of a thromboplastic enzyme, in addition to the phospholipid factor and acting via cephalin mobilization, offers a solution to a wide variety of coagulation problems, including the clotting delay in hemophilia (8).

SUMMARY

With the recognition of the fact that the clotting activity of trypsin is dependent upon calcium, it becomes possible to institute *direct* comparisons of the enzyme with a major, somewhat unstable, and non-lipid component of plasma prothrombin and aqueous tissue extracts.

The experimental analysis reveals that a "thromboplastic enzyme" may be postulated which, under ordinary conditions of thrombin formation, contributes to the mobilization of the essential phospholipid factor. Thrombin formation may be conceived of as an enzymic mobilization of cephalin and calcium at the colloidal surface of the protein (prothrombin) substrate, with the resulting elaboration of a substance capable of clotting fibrinogen.

The postulated amounts of enzyme are too small to evince gross proteolysis, but syneresis, fibrinolysis and thrombinolysis may be attributed to the feeble digestive powers of the trypsin-like "thromboplastic enzyme."

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³ Owing to this thrombinolysis, prothrombin-free tissue extracts result from simple keeping and do not require the cumbersome and diluting technique of perfusion (cf. 20).

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THE CLOTTING OF HEMOPHILIC PLASMA BY THROMBOPLASTIC ENZYME

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It has recently been shown that the clotting power of crystalline trypsin (4), in small concentrations, is dependent upon the presence of ionized calcium and aided by addition of cephalin (2). The ability of trypsin to clot hemophilic blood was recorded by Tyson and West (6) without data, other than a comparison with placental extract, to throw any light upon its mode of action.

The tests of table 1 confirm the ability of suitable concentrations of trypsin to aid in the clotting of recalcified citrated hemophilic plasma.¹ The two cases cited represent extremes of a number which have been studied. With simple recalcification the typical delay in coagulation (cf. normal:3-5 min.) is noted. The effect of adding cephalin will be discussed in a subsequent paragraph.

Trypsin in too large an amount (1, 11) causes thrombolysis, which interferes with the early development of a good clot but a coagulum which partially resists lysis may appear and persist some hours later. Too weak an enzyme concentration, unassisted, fails to evoke clotting. Calcium is a powerful synergist to the enzyme action. With the larger amounts of trypsin, clotting is hastened and improved if calcium is present. The significant finding, however, is that amounts of enzyme (e.g., 0.1 mgm.), which are too small to be effective alone, induce very rapid, firm and *permanent* clots in the presence of calcium. Cephalin in addition to calcium provides optimal conditions and in every plasma tested with this mixture the clotting time became a matter of seconds!

We have previously shown (2) that trypsin acts as a thromboplastic agent in the activation of prothrombin. In a series of researches initiated by Patek and Stetson (5), a "globulin substance" (essentially a Mellanby-type "prothrombin"), prepared by the careful acidification to pH:6.0 of diluted hemophilic plasma, showed at least a quantitative defect in serving as a coagulant for hemophilic blood. We have prepared "globulin

¹ We are indebted to Dr. P. Lee of the Children's Hospital, Detroit, for supplies of authenticated hemophilic blood and plasmas.

substance" from several hemophilic plasmas and find it to be very markedly improved in regard to the rate and amount of thrombin yielded on recalcification in the presence of trypsin, especially under optimal conditions of calcium and cephalin.

The experiments of table 2 demonstrate the effect of trypsin on the process of thrombin formation in recalcified "globulin substance" from the plasma of an authenticated hemophiliac. Although the clotting delay (11-12 min.) was not very marked in this case, the restoration of the

TABLE 1

Effects of calcium and cephalin on clotting of citrated and heparinized hemophilic plasmas by crystalline trypsin (controlled dilutions)

| | HEMO- PHILIC PLASMA | DISTILLED WATER | CEPHALIN 1:1000 | CaCl ₂ N/10 | CRYSTALLINE TRYPSIN IN 0.2 CC. | CLOTTING-TIMES (SEC.) AT 35°C. | |
|----|---------------------------|--------------------|--------------------|---------------------------|-----------------------------------|-----------------------------------|---------|
| | | | | | | A | B |
| | cc. | cc. | cc. | cc. | mgm. | | |
| 1 | 0.5 | 0.3 | | 0.2 | | 720" | >7 hrs. |
| 2 | 0.5 | 0.2 | 0.1 | 0.2 | | 340" | >7 hrs. |
| 3 | 0.5 | 0.3 | | | 0.1 | ∞ | |
| 4 | 0.5 | 0.2 | 0.1 | | 0.1 | ∞ | |
| 5 | 0.5 | 0.1 | | 0.2 | 0.1 (0.02) | 65" | (1020") |
| 6 | 0.5 | | 0.1 | 0.2 | 0.1 | 36" | |
| 7 | 0.5 | 0.3 | | | 0.4 (0.5) | 120" | (180") |
| 8 | 0.5 | 0.2 | 0.1 | | 0.4 | 107" | |
| 9 | 0.5 | 0.1 | | 0.2 | 0.4 (0.5) | 27" | (60") |
| 10 | 0.5 | | 0.1 | 0.2 | 0.4 (0.5) | 17" | (22") |
| 11 | 0.5 | 0.3 | | | 1.0 | † | † |
| 12 | 0.5* | 0.2 | 0.1 | 0.2 | | | ∞ |
| 13 | 0.5* | 0.1 | | 0.2 | 0.5 | | 120" |
| 14 | 0.5* | | 0.1 | 0.2 | 0.5 | | 100" |

† Thrombolysis: trace of persistent clot overnight.

thrombin formation process to well within the normal by the addition of a weak trypsin solution is none-the-less convincing.

It may be concluded that, in so far as the complete in vitro restoration, both qualitative and quantitative, of the defective clotting function by a pure chemical factor constitutes evidence for the cause of the deficiency, this criterion has been fulfilled in the case of hemophilia.

In postulating this enzyme defect, it is essential to state why thrombin formation cannot proceed without the factor in question. A reasonable explanation is to hand (1) in the "cephalin availability theory" which we first advanced in this Journal in 1936. This theory is based upon the

contention, for which adequate proof is steadily accumulating (2), viz., that the phospholipid, which is an essential component of the reaction of thrombin formation, requires the action of a weak proteolytic enzyme, like trypsin, to mobilize it from the normally inactive protein combinations.

Cephalin in hemophilic plasma. At first sight it would appear that the simple addition of cephalin should, on this theory, correct for the defect in hemophilia. The facts do not show any general consistency in the ability of cephalin to accelerate the clotting of hemophilic blood. We have made analytical studies, however, which reveal that 1, whereas theoretical P-lipid values are obtainable on ether extracts of dried cephalin

TABLE 2

Activation of recalcified hemophilic "globulin substance" (prothrombin) by crystalline trypsin

G₁ = 2.0 cc. hemophilic "globulin substance" (G) + 0.4 cc. distilled water + 1.6 cc. N/10 CaCl₂.

G₂ = 2.0 cc. hemophilic "globulin substance" (G) + 0.4 cc. (= 0.1 mgm.) trypsin + 1.6 cc. N/10 CaCl₂.

Mixtures held at 24°C.; 0.25 cc. test portions added to 0.5 cc. quantities of hemophilic citrated plasma (same case) at times shown. Clotting-times, seconds, at 38°C.

| | THROMBIN FORMATION PERIOD, MINUTES, AT 24°C. | | | | | |
|----------------------|--|------|------|------|------|-----|
| | 1' | 5' | 10' | 20' | 30' | 45' |
| G ₁ | 270" | 180" | 145" | 135" | 105" | 62" |
| G ₂ | 40" | 17" | 10" | 7" | 7" | 7" |

Controls: 1. 0.5 cc. hemophilic plasma + 0.3 cc. distilled water + 0.2 cc. N/10 CaCl₂ = 720" (C.T.)

2. 0.5 cc. hemophilic plasma + 0.1 cc. H₂O + 0.2 cc. 'G' + 0.2 cc. N/10 CaCl₂ = 275"

3. 0.5 cc. hemophilic plasma + 0.3 cc. H₂O + 0.2 cc. (= 0.1 mgm.) trypsin = no clot

4. 0.5 cc. hemophilic plasma + 0.2 cc. 'G' + 0.2 cc. CaCl₂ + 0.2 cc. trypsin = 70"

solutions; 2, similar procedures fail to extract either the initial content of P-lipid or considerable quantities of added cephalin, when determinations are made on normal or hemophilic plasma. These data confirm the above contention (2) as to phospholipid-protein combinations and also demonstrate the ability of proteins to combine with and "immobilize" added free cephalin.

The tendency for clot-acceleration with cephalin is greater, the milder the case in terms of the delay in coagulation. Recalcified enzyme works equally well, whatever the severity. It would appear that added cephalin is of some assistance when there is enough enzyme to take advantage

of it. In the absence of the mobilizing effect of the enzyme, the availability of the cephalin is nullified by inert combinations with the plasma proteins.

Heparinized hemophilic plasma. Recalcified trypsin, in marked contrast to cephalin plus calcium, can clot hemophilic plasma which is well preserved with heparin. The significance of the failure of the "antiproteolytic" effect of heparin in the presence of thromboplastic enzyme will be fully dealt with in a forthcoming communication.

DISCUSSION. The status of our knowledge concerning hemophilia has recently been reviewed by Howell (3) in a paper containing an important series of preliminary data pointing to the lack of a thromboplastic factor in this disease. Believing that the preparations of this author might still contain significant traces of proteolytic enzyme in too high a dilution to respond to the chemical tests for enzyme protein (cf. 4), we have made careful tests which show that *a*, the *biuret test* ceases to detect Northrop and Kunitz's trypsin between concentrations of 0.4 and 0.2 mgm. per cubic centimeter, and *b*, the *ninhydrin reaction*, tested during incomplete solution of trypsin, is only positive around the grains of undissolved enzyme. There is also the possibility that Howell is dealing with the enzyme activator (*kinase*) of Schmitz (1937).

SUMMARY

By the addition of pure crystalline trypsin, optimally potentiated by calcium (and cephalin), it is possible to activate the prothrombin of hemophilic "globulin substance" to normal thrombin and to coagulate hemophilic plasma (in vitro) in manner and extent indistinguishable from the behavior of normal plasma.

These facts, together with the contention, for which we have accumulated considerable evidence, viz., that phospholipids are normally in firm combination with proteins and, ordinarily, require a trypsin-like enzyme to make them "available" for an essential rôle in thrombin formation, support the conclusion that a deficiency in the amount of "thromboplastic enzyme" in the *plasma* is a logical explanation for the coagulation delay of hemophilia.

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LOCALIZATION OF THE MEDULLARY RESPIRATORY CENTERS IN THE CAT¹

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Beginning with the work of Longet (1847) there has accumulated an impressive mass of evidence which indicates that the indispensable mechanism for the neural control of respiration is localized in the reticular formation of the caudal half of the medulla (Brown-Sequard, 1860; Gad and Marinesco, 1893; Bechterew, 1885; Kohnstamm, 1900; etc.). Although there have appeared sporadic assertions that isolated structures play a predominant rôle in respiratory control, e.g., the *ala cinerea* (Schiff, 1870; 1871), the *tractus solitarius* (Gierke, 1873), the central nuclei of the medullary raphé (Mislowsky, 1885), recent reviews of the subject by Finley (1931) and Cordier and Heymans (1935) adequately demonstrate the preponderant importance of the medullary reticular formation.

A functional subdivision of the respiratory center into inspiratory and expiratory portions has been attempted by Marekwald and Kronecker (1880), Lewandowsky (1896), Mislowsky (1885) and Gad and Marinesco (1893). Lumsden (1923) claims to have distinguished 4 centers, a pneumotaxic, an apneustic, an expiratory and a gasping center. Henderson and Sweet (1929) maintain that only 1 respiratory center exists.

Attempts at more definite localization within the reticular formation by the transection, partial section, isolated destruction and crude stimulation methods of the early investigators have succeeded only in delimiting the cephalic limits of the respiratory center as somewhere caudal to the entrance of the eighth nerves. Gesell, Bricker and Magee (1936) in a study of amplified potentials showing a respiratory rhythm tapped from the brain stem place the cephalic limit of the respiratory center within the reticular formation at the level of entrance of the upper vagal rootlets and the cephalic end of the inferior olive. The caudal limit they place 2 mm. below the obex at the level of the decussation of the pyramids.

In view of the indefinite localization of the medullary respiratory center and the disagreement as to its functional subdivision it seemed to us that a careful study of the respiratory responses to stimulation of the brain stem

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of the cat utilizing the Horsley-Clarke technique as described by Ranson (1934) might lead to more accurate knowledge concerning the site, extent and functional significance of the respiratory center.

METHODS. This investigation was carried out on cats anesthetized for the most part with 15 to 25 mgm. of nembutal per kilo body weight injected intravenously and supplemented with ether during operative procedures. Additional observations were made in animals under ether anesthesia, in others decerebrated under ether and allowed to recover from the effects of the anesthesia, and in normal unanesthetized animals in which electrodes had been inserted into the respiratory center and sealed in the skull some 3 to 5 hours previously under ether anesthesia. The calvarium was trephined and rongeured away to provide proper exposure. Bipolar electrodes of enameled nichrome wire were used for stimulation, the tips being ground to a point and separated about 0.2 mm. along the axis of the electrode. The electrodes were fixed in the carrier of the Horsley-Clarke stereotaxic instrument, which in experiments where stimulation was carried out caudal to the tentorium, was tilted 15° forward from the verticle (Harrison, 1938), to permit more convenient exploration of this region. All points stimulated were identified in Weil stained serial sections cut in the plane of the punctures. Stimulating current was obtained from the thyatron regulated discharge of a 0.1 mfd. condenser coupled to the leads by an air core transformer. Frequencies and voltages given are accurate to well within 10 per cent as shown by oscillographic calibration with electrodes in the brain. Respiration was recorded by cannulating the trachea and attaching to the cannula a small spirometer built in the form of the familiar Krogh basal metabolism machine. Since the system is a closed one carbon dioxide was absorbed by a soda lime tube placed next to the tracheal cannula to minimize dead space and oxygen was added as needed to maintain volume. It must be emphasized that this system of recording respiration is the only one tried that is at all suitable. A tracheal cannula recording through a tambour will obviously record only changes in rate and gives no indication of tonic changes in respiratory midposition. Thoracic pneumographs though accomplishing the latter are entirely unreliable since they record torsions and movements of the body which may result from stimulation. The spirometer used is entirely unaffected by general body movements. In the kymograph records presented, the slope of the record inherent in a closed system intermittently refilled has been eliminated by trimming the records in the interest of space conservation. All time markings are at 6 second intervals. For routine exploration a stimulus intensity of 8 volts and 240 stimuli per second has been used for reasons which will appear in the text.

OBSERVATIONS. *General types of reactions.* It became evident to us in our early experiments that there could be obtained from the medulla with

relatively low stimulus intensity striking respiratory responses far surpassing anything obtainable from higher regions of the brain stem. This paper will deal solely with these medullary reactions. Two striking types of response were noted, the one a deep inspiration tonically maintained for the duration of the stimulus (fig. 1A and B); the other an expiration similarly tonically maintained (fig. 1E, F and G). In both instances the stimulus used was 8 volts and 240 stimuli per second. In both types of response it is apparent that apnea is maintained uninterrupted by any rhythmic respiration for the duration of the stimulus (12 sec. to 3 min.). The inspiration in figure 1A amounts to 200 cc. whereas the tidal air immediately preceding stimulation was only 25 cc. The appearance of the animal during this inspiration is striking, the chest appearing to be maximally expanded. The expirations shown in figure 1E, F and G are considerably less dramatic both in appearance of the animal and in the amount of air expired but it is worthy of note that it is often impossible to deflate the chest appreciably more by firm compression with the hand (fig. 1E, note the 3 small deflections caused by such compression). However, in figure 1G is represented a common type of response where expiration seems little more than that seen in normal respiration. Some animals showed only this type of response and since there are intergrades (fig. 1F), it was felt that they were essentially similar. These responses, maintained excessive inspiratory apnea and maintained expiratory apnea, were soon found to be obtainable only from definitely localized regions; and a thorough exploratory stimulation of the pons, medulla and upper cervical cord was performed to delimit accurately the extent of these regions.

Many varieties of respiratory responses were obtained. In fact if one considers all the possible combinations of increases, decreases and no change, in rate, amplitude, midposition etc., each can be found in the medulla and an attempt to indicate localization would lead to confusion and detract from the more precise localization of maximal reactions. Consequently we have chosen to consider the 2 types of response shown in figures 1A, B and 1E, F, G as maximal types if the apnea maintains without interruption for the duration of the test stimulus, i.e., 12 to 18 seconds or more and if, in the case of inspiration the overinspiration amounts to 75 cc. or more, and if in the case of expiration the position of rest is in at least as great a degree of expiration as that of normal respiration preceding stimulation. Lesser reactive degrees are grouped into 3 classes, those showing slighter degrees of inspiratory apnea or only a shift in midposition of the respiratory cycle toward the inspiratory side, figure 1C and D; those showing similar expiratory effects (fig. 1H) and those showing apnea in midposition or diminution of amplitude in midposition (fig. 1, I and J). Very slight changes have been entirely disregarded.

It must be emphasized that with a stimulus intensity of 8 volts at 240

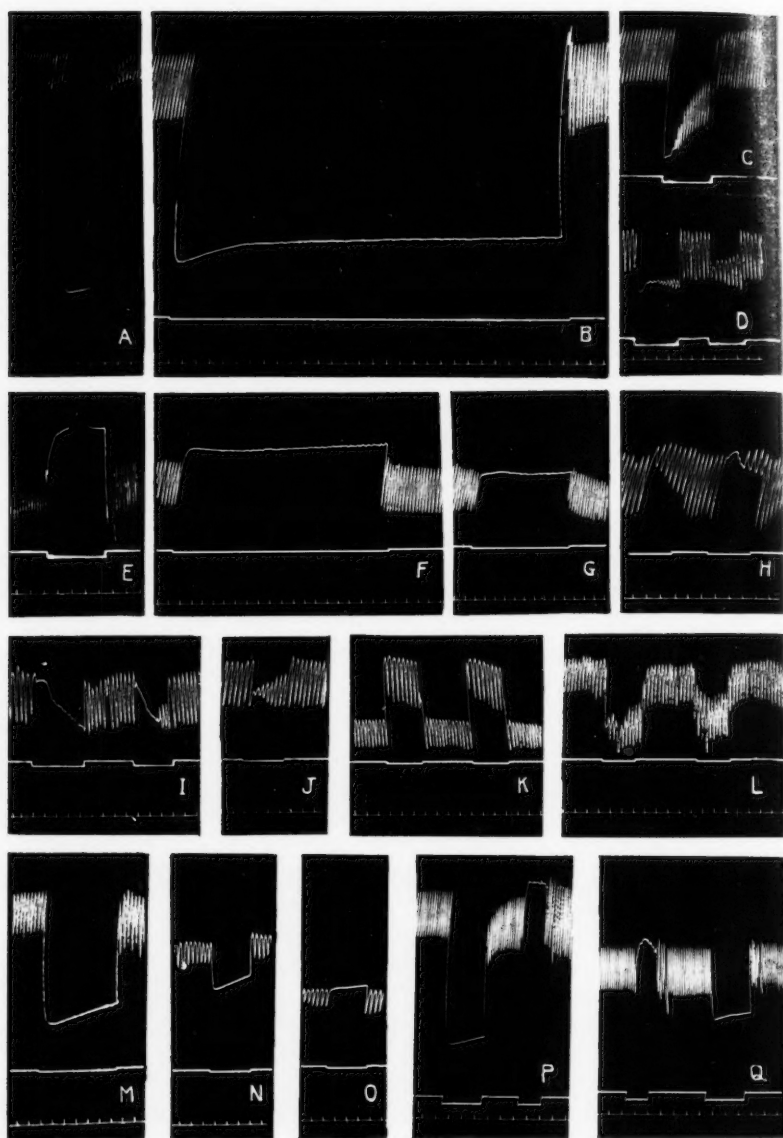


Fig. 1. Spirometer tracings of respiratory reactions to stimulation of the medulla. In all records a constant strength of stimulus was used, 8 volts at 240 stimuli per second. The duration of stimulation is indicated by the signal magnet. Time intervals are 6 seconds. Inspiration is the downstroke, expiration the upstroke. A and B, maximal inspiratory reactions; C and D, submaximal inspiratory reactions; E, F and G, maximal expiratory reactions; H, submaximal expiratory reactions; I and J, apnea and reduction in amplitude in midposition; K and L, specific types of submaximal expiratory and inspiratory responses obtained on stimulation of the lower end of the medulla at the level of decussation of the pyramids; M, a control inspiratory response before, and N and O, inspiratory and expiratory responses after complete section of the spinal cord at C_7 ; P, control inspiratory and expiratory reactions before and Q, same reactions after bilateral section of the phrenic nerves.

stimuli per second there is little spread of current beyond the tips of the electrodes. In fact we routinely observed in certain regions a complete reversal of response from expiration to inspiration on moving the tips of the electrodes 1 mm.

Localization. In figures 3A to D and figures 4E to H are drawings of sections of the medulla taken at 1 mm. intervals from 2 mm. above the cephalic end of the inferior olive to 1 mm. below the caudal end of the inferior olive. Between sections G and H is an interval of 2 mm. The reactive points which have all been identified in serial Weil stained sections of the medullas have been grouped according to level and plotted on these figures to the nearest $\frac{1}{2}$ mm. The inferior olive is constant enough in its extent to serve as an accurate marker for placing our levels of stimulation in the proper grouping. Thus it is possible by counting sections (50 μ) to place any point of stimulation a given number of millimeters from either the cephalic or caudal end of the inferior olive. The solid triangles represent maximal inspiratory responses, the solid circles maximal expiratory responses. The hollow figures represent submaximal inspiratory and expiratory responses while the plus marks indicate midpositional apneas or reductions in amplitude.

In figure 2 the location of the maximal inspiratory and maximal expiratory responses is diagrammatically shown projected on a surface view of the fourth ventricle. To avoid overlapping, the expiratory reactive region is indicated only on the left, inspiratory on the right. The locations of the sections shown in figures 3 and 4 are indicated on the side. It may be seen that in figure 3B the first maximal expiratory responses appear just above the facial nucleus. In figure 3C they appear in fullest extent, occupying the dorsal reticular formation from the midline to the spinal tract of the trigeminal. They may be followed through sections D and E where they lie in the dorsal part of the reticular formation in the same relative position and then disappear quite suddenly. The maximal inspiratory responses begin in the section illustrated in figure 3C in the ventral reticular formation just dorsal to the cephalic end of the inferior olive and continue in the same relative position expanding to greatest proportions over the middle of the inferior olive in sections 3D and 4E and F. They disappear about 1 mm. above the caudal end of the olive.

The location of these maximal inspiratory and expiratory responses can be briefly summarized as being in the reticular formation of the medulla in the region over the inferior olive, the expiratory region lying dorsal to, slightly cephalic to, and cupped over the cephalic end of the inspiratory region which itself lies as a plate over the dorsal surface of the upper four-fifths of the inferior olive. Rough calculations indicate that the inspiratory and expiratory regions occupy each about 30 cu. mm. of the reticular formation. It must be emphasized that these maximal responses are

obtained from no other region of the brain stem than that shown in figures 3B, C, D and 4E, F, G. Our exploration has extended from a level in front of the hypothalamus to the cervical cord. Similar emphasis should be placed on the relatively sharp and constant boundaries of these respiratory regions which we have defined in all dimensions. Though the experi-

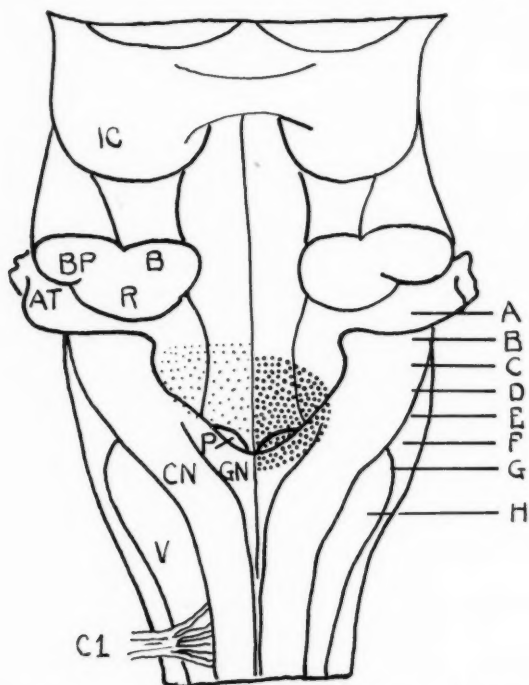
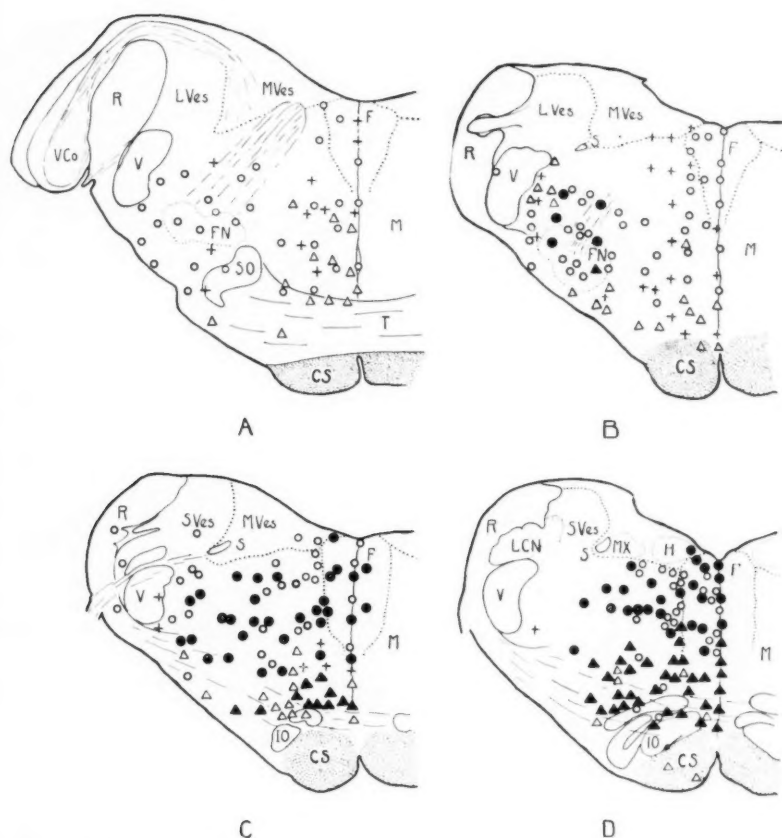


Fig. 2. Dorsal view of the lower brain stem of the cat with the cerebellum removed. The location of the maximal inspiratory (heavy stippling) and maximal expiratory (light stippling) responses is diagrammatically shown projected on the floor of the fourth ventricle. To avoid overlapping, the expiratory reactive region is indicated only on the left, inspiratory only on the right. On the right side of the figure the lettering from A to H indicates the level of the sections shown in figures 3 and 4. Acoustic tubercle, *AT*; brachium conjunctivum, *B*; brachium pontis, *BP*; first cervical dorsal root, *C₁*; cuneate nucleus, *CN*; gracile nucleus, *GN*; inferior colliculus, *IC*; area postrema, *P*; restiform body, *R*; tuberculum cinereum, *V*.

ments were not designed in such a way that exactly the same number of points were stimulated at each level shown in figures 3 and 4, actually the data do not fall far short of attaining this end, and thus the number of points shown roughly indicates the relative reactive density.

The significance of the minor responses in figure 3A and B above the



Figs. 3 and 4. Sections of the medulla of the cat taken at 1 mm. intervals from 2 mm. above the cephalic end of the inferior olive to 1 mm. below the caudal end of the inferior olive. Between sections G and H is an interval of 2 mm. The plane of the sections is sloping downward and forward 15 degrees from the verticle coronal plane of the Horsley-Clarke coordinates. The relation of these sections to surface anatomy of the medulla is shown on the right side of figure 2. Solid circles, maximal expiratory reactions; solid triangles, maximal inspiratory reactions; hollow circles, submaximal expiratory reactions; hollow triangles, submaximal inspiratory reactions; plus signs, midpositional changes, i.e., reductions in amplitude or apnea. Cuneate nucleus, CN; corticospinal tract, CS; medial longitudinal fasciculus, F; gracile nucleus, GN; hypoglossal nucleus, H; intercalate nucleus, I; inferior olive, IO; lateral cuneate nucleus, LCN; lateral reticular nucleus, LN; lateral vestibular nucleus, L ves; medial lemniscus, M; motor nucleus of vagus, MX; medial vestibular nucleus, M ves; area postrema, P; restiform body, R; tractus solitarius, S; spinal vestibular tract and nucleus, S ves; spinal tract of the trigeminal nerve, V; ventral cochlear nucleus, V Co.

lower end of the medulla. Exploration was performed to a level of the first cervical nerve but since identification of the punctures was impossible due to small size and curvature of the cord no levels lower than that shown in figure 4H can be shown. Qualitatively this same distribution of inspiratory and expiratory responses was noted at the time of such experiments though subsequent identification of punctures was impossible.

A consideration of the results presented so far suggests that the region of maximal inspiratory and maximal expiratory responses is the same as that which has been termed the respiratory center by preceding workers in the field. Such a view is justified by the consideration of the following points which are here stated briefly but will be discussed more in detail later. These responses are coordinated respiratory acts. They are probably not due to stimulation of afferent pathways to or efferent pathways from the respiratory centers. They are invariable responses only quantitatively influenced by the strength or frequency of stimulation. These responses are dominant over respiratory and vascular reflexes. They are independent of anesthesia. Coordinate respiration may be attained by rhythmic stimulation of these centers. Thus throughout the rest of the paper we feel justified in using the term respiratory center to describe the regions we have localized in the preceding paragraphs as yielding maximal inspiratory and maximal expiratory responses.³ For convenience we shall use the terms *inspiratory center* and *expiratory center* though the latter might be more fittingly called an inspiratory inhibitory center. By using the term "center" we mean that the region of the reticular formation which we have localized above, undoubtedly containing many neurons richly interconnected synaptically, acts as a supranuclear regulating, coordinating and driving mechanism for the lower phrenic and thoracic cord nuclei.

Coordination of thorax and diaphragm. The magnitude and persistence of the maximal inspiratory and expiratory apneas obviously suggest that the diaphragm as well as the thorax participates in the reactions. In fact with the abdomen opened powerful tetanic diaphragmatic contractions could be seen on stimulation of the inspiratory center, whereas relaxation was evident on stimulation of the expiratory center. In figure 1M is shown an inspiratory response prior to section of the cervical cord at C₇, and in figure 1N and O are inspiratory and expiratory responses after section, only the diaphragm being active. In figure 1P are shown inspiratory and expiratory responses before bilateral phrenic nerve section, and in figure 1Q the same responses after nerve section, only the thoracic

³ The reactions which Monnier (1938) obtained on stimulation of the dorsal and ventral reticular formations of the medulla of the cat and which he classified as postural hypertonia of the expiratory muscles with apnea and postural hypertonia of the inspiratory muscles with apnea, are undoubtedly the same as those described here as obtained from the respiratory centers.

musculature being active. It is evident that the responses are the same though quantitatively reduced if either thorax or diaphragm is eliminated, and thus that the stimulation of either the inspiratory or expiratory center gives a coördinated respiratory response.

Elimination of afferent pathways. The possibility that we are stimulating afferent fibers to the respiratory centers rather than the centers themselves is a valid criticism. We have attempted to rule out such a possibility in the following ways. Stimulation of the brain stem either above or below the center does not yield the typical maximal responses (see fig. 3A, B and fig. 4H). Thus stimulation of ascending or descending fiber tracts alone will not yield the response. The possibility exists that these responses are so scattered both above and below the region where maximal responses are obtained, that only a fraction of the fibers are stimulated and hence the reactions are of lesser magnitude. This is ruled out in a number of ways. The centers themselves are not as circumscribed as one would expect a compact bundle of afferents or efferents to be. Furthermore the typical inspiratory and expiratory responses are obtained both ipsilaterally and contralaterally above a chronic hemisection of the spinal cord at C_1 . These same responses are obtained on both sides below a chronic medullary hemisection at the level of the acoustic tubercles. In these animals stimulated 4 to 8 weeks after operation afferent or efferent tracts passing through this region would have degenerated. Furthermore these experiments demonstrate rich crossed connections at the level of the centers. The maximal inspiratory and expiratory reactions are in no way similar to results obtained on stimulation of an afferent pathway such as the sciatic nerve which usually causes reflex hyperpnea. Stimulation of the central end of the vagus gives highly variable results, in no case approaching the results of stimulation of the inspiratory center. In some cats an expiratory apnea does result from central vagal stimulation not unlike that obtained from the expiratory center. However, a glance at figures 3 and 4 indicates that the extent of the expiratory center is not that of the entering vagal fibers or their secondary sensory connections in the nucleus of the tractus solitarius. Evidence to be presented in a succeeding paper does, however, indicate rich central connections of the vagus with the expiratory center in its function of pneumotaxis.

Magnitude of response in relation to extent of stimulus. The magnitude of the maximal responses obtained is worthy of emphasis. The inspiration on stimulation of the inspiratory center may amount to over 200 cc., often over 10 times the tidal air. This response is obtained by stimulation of less than 3 cu. mm. of the reticular formation, for with voltages as high as 20 volts spread of stimulus is less than 1 mm. judging from reversal or disappearance of reaction on moving the electrodes 1 mm. Since the

volume of brain substance from which maximal responses may be obtained is over 30 cu. mm. and since the direct excitation of less than 3 cu. mm. within this region can yield responses which appear to be maximal, it is probable that the center is fired as a whole through rich synaptic interconnections. Evidence to be presented in a succeeding paper confirms this view.

Variation of voltage and frequency of stimulation. In figure 5A and B is shown an experiment indicating the effects of variation in voltage and frequency on the activity of the inspiratory center. In figure 5A a constant frequency of 240 stimuli per second was used at strengths from 1 to 15 volts. A perceptible inspiratory hypertonus is seen with 1 volt, apnea appears at 3 volts and at 6 volts is maintained. At 8 volts the response appears maximal. In figure 5B a constant stimulus intensity of 8 volts was used and frequency varied as shown from 240 to 34 stimuli per second. It is seen that the higher frequency stimuli are more effective. It should be emphasized that only one condenser, a 0.1 mfd. one was used throughout the voltage and frequency range so that the form of the stimulus should remain essentially constant. Other frequency series were run at both higher and lower voltages with qualitatively similar results. At lower voltages higher frequency was necessary to maintain maximal apnea. It is evident that an increase in frequency makes up to some extent for a decrease in voltage and since it is desirable to keep the stimulus as localized as possible a value of 8 volts and 240 stimuli per second was chosen for routine exploratory stimulation. In figure 5C and D similar variations of voltage and frequency yielded qualitatively similar results on stimulation of the expiratory center. It may be seen that the responses obtained with the low stimulus intensities are not dissimilar to some of the responses shown in figure 1C, D and H as submaximal responses. It is equally evident that the inspiratory center yields only inspiratory hypertonias of varying degree and the expiratory center yields only expiratory hypertonias independent of stimulus strength or frequency within the limits specified and available to us. There has never been any suggestion of response reversal.

Independence of respiratory and vascular responses. In figure 5E and F are shown simultaneous records of respiratory responses and blood pressure with stimulation of the inspiratory and expiratory center. It is evident that minimal cardiovascular effects result from stimulation of the centers. What little variation is present may be explained on the basis of mechanical obstruction or as responses to increased carbon dioxide tension within the lungs. If the spirometer is filled with air rather than oxygen a typical asphyxial rise in blood pressure occurs followed by cardiac slowing, irregularity and fall in blood pressure. A consideration of figures 3 and 4 shows

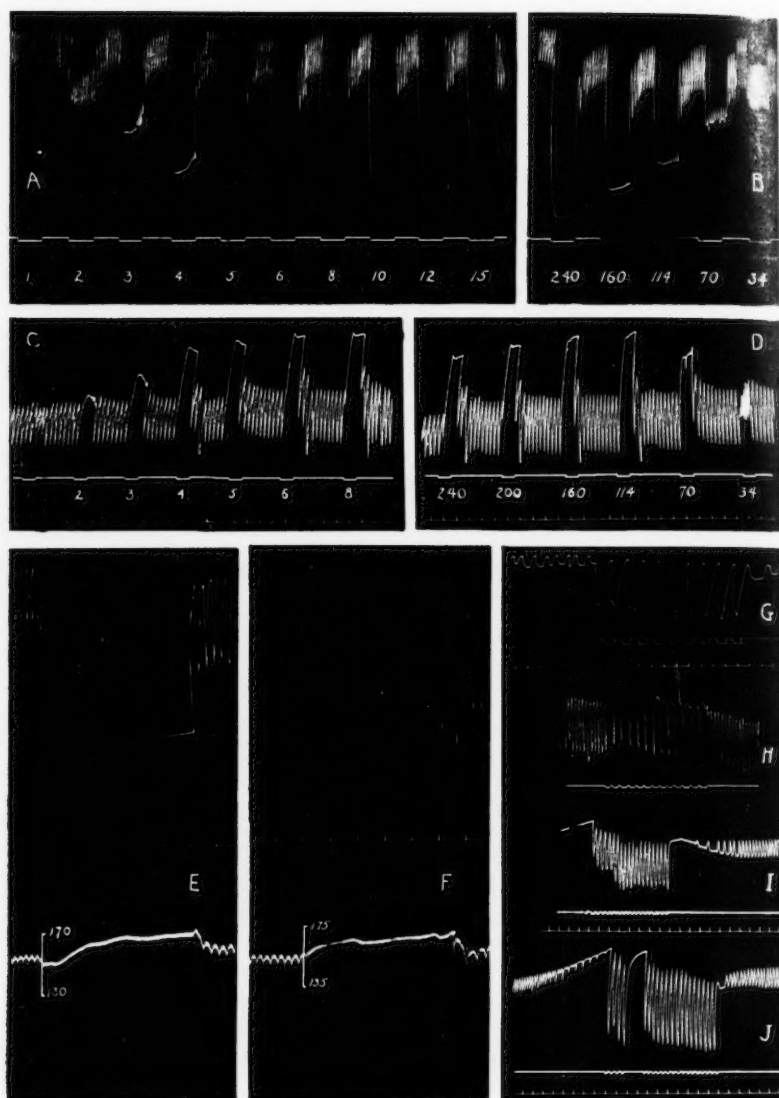


Fig. 5. Spirometer tracings of respiratory reactions and mercury manometer tracings of cardiovascular reactions to stimulation of the medullary inspiratory and expiratory centers. A to D, comparisons of the effects of alteration in voltage and frequency on the respiratory responses elicited from stimulation of the inspiratory center, A and B, and the expiratory center, C and D. In A and C the frequency was maintained constant at 240 stimuli per second and the voltage varied as shown on the record. In B the voltage was maintained at 8 volts and in D at 6 volts and the frequency varied as indicated on the record. In E and F simultaneous records of respiration and blood pressure during stimulation of the inspiratory and expiratory centers are shown. In records G to J coordinate respiratory movements were induced by rhythmic stimulation of the inspiratory center, G; expiratory center, H; inspiratory center after overanesthesia with ether, I; and in a deteriorating animal, J.

that the region of the respiratory centers differs considerably in extent and location from that which yields significant cardiovascular responses (Wang and Ranson, 1939).

Coördinate respiration produced by stimulation of the centers. In figure 5G the inspiratory center of an animal which had a rather slow irregular respiration was stimulated rhythmically with stimuli of about 4 seconds duration. It is seen that respiration is converted into a slow smooth regular sequence. Expiration is passive and the next inspiratory stimulus is introduced prior to the beginning of the next spontaneous inspiration. In figure 5H respiration is controlled by rhythmic stimulation of the expiratory center. Here inspiration is spontaneous and the expiratory stimulus is introduced prior to the beginning of the next spontaneous expiration. In figure 5I is shown the record of an animal overanesthetized with ether to

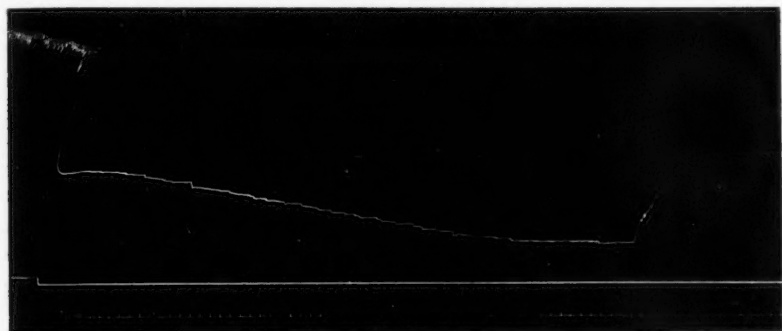


Fig. 6. Spirometer tracing of the respiratory reaction to continued stimulation of the inspiratory center to death. Time 6 second intervals. Stimulus 8 volts at 240 stimuli per second. The irregularities in the record result from asphyxial convulsive activity.

cessation of respiration. The inspiratory center was rhythmically stimulated for a time and on cessation of the stimulation normal respiration was resumed. In figure 5J is the record of an animal deteriorating as a result of shock and excessive medullary puncturing. Rhythmic stimulation of the inspiratory center was followed by a return of spontaneous respiration and the animal lived for several minutes more.

Dominance of the respiratory reactions. Figure 6 is the record of stimulation of the inspiratory center of an animal continuously till death. Inspiration is maintained deep and uninterrupted for a period of 6 minutes. At the end of this time irritability of the inspiratory center was lost and gradual passive expiration occurred. The heart at this time was slow and irregular and ceased beating a few minutes later. It is evident that inspiration is maintained without interruption. The irregularities in the record

result from asphyxial convulsive activity. If air rather than oxygen is used in the spirometer death occurs after 2.5 to 3 minutes and the mucosae are markedly cyanotic. In figure 6 the stimulus to breathe must certainly have been maximal yet apnea to death was maintained. We have not been able to maintain apnea in expiration for longer than 3 minutes, never till death. Spontaneous inspirations break through after a time and permit sufficient ventilation to maintain life. As pointed out before, the expiratory center might better be called an inspiratory inhibitory center, and with such a strong stimulus to inspire as asphyxia, inhibition of inspiration fails.

Anesthesia. Essentially identical results on stimulation of the inspiratory and expiratory centers have been obtained under nembutal anesthesia using from 15 to 40 mgm. per kilo body weight given intravenously, under ether anesthesia and in animals decerebrated under ether and allowed to recover. Decerebrate animals are less resistant to repeated medullary puncture with the electrode and deteriorate more rapidly than those anesthetized with nembutal. A most interesting type of experiment was performed by placing electrodes in the inspiratory center and sealing them firmly in the skull with dental cement. From 3 to 5 hours after discontinuation of the ether and at a time when the animal was walking around the room, the inspiratory center was stimulated with 2 volts at 240 stimuli per second. A perceptible overinspiration was noted lasting for 2 to 3 seconds. With 5 volts an apparently maximal inspiratory apnea was maintained for 15 seconds followed by shallow respiratory excursions superimposed on a maintained inspiratory hypertonus. With 10 volts a maximal inspiratory apnea was maintained for 1 minute 15 seconds with a few spasmodic shallow respirations then appearing on the inspiratory hypertonus. On cessation of stimulation the animal showed a marked hyperpnea. With 20 volts the animal maintained maximal inspiratory apnea to death which occurred after 3 minutes of stimulation.

SUMMARY

In the preceding paragraphs we have defined a localized region of the ventral reticular formation of the medulla of the cat immediately overlying the cephalic four-fifths of the inferior olive, from the stimulation of which may be obtained maximal inspiration involving both thorax and diaphragm. The chest and diaphragm remain fixed in maximal inspiration and rhythmic respiration is abolished. The inspiration may be maintained to death by continued stimulation. Dorsal to, slightly cephalic to and cupped over the cephalic end of the inspiratory reticular formation is a region of the dorsal reticular formation from the stimulation of which may be obtained expiration involving both thorax and diaphragm (in the latter passive relaxation) which in some instances is maximal. In some animals only a

passive expiration is obtained but in either instance apnea may be maintained in a passive to a maximally active expiratory position for a period up to 2 or 3 minutes, inspiration then gradually breaking through. We have presented evidence that these regions comprise the respiratory center of previous workers in the field of the neural control of respiration. These lines of evidence include the following. The respiratory responses are well localized within the region which previous workers have vaguely defined as the respiratory center. Responses are maximal coordinated respiratory acts. The responses are probably not due to stimulation of afferent or efferent fiber tracts but to stimulation of a neuron field closely interrelated synaptically. They are invariable responses only quantitatively influenced by strength and frequency of stimulation. They are independent of the anesthetic used and may be obtained in unanesthetized animals with electrodes sealed in the skull.

The cephalic and caudal limits which we have defined above for the respiratory center in the cat agree quite well with the limits defined by Gesell, Bricker and Magee (1936) for the respiratory center in the dog.

Functionally the respiratory center is divided into an inspiratory and an expiratory center, the latter so called for simplicity though it would better be termed an inspirato-inhibitory and expiratory center. Caudal to these centers which extend to slightly below the obex we have defined responses which consist of an inspiratory or expiratory hypertonus with superimposed rhythmic respiration little altered in rate or depth from the normal. These responses we feel are obtained from the descending pathways from the inspiratory and expiratory centers to the lower phrenic and thoracic cord nuclei.

This localization of descending respiratory pathways agrees with observations of Rothmann (1902) and Allen (1927) which indicate that the descending respiratory pathways in the spinal cord lie in the anterior columns and in the ventral part of the lateral columns.

That this location of the respiratory centers in the cat may be applied roughly to man is indicated by figures presented by Finley (1931) showing lesions in the medullary reticular formation overlying the inferior olive from 2 cases of neurologic respiratory failure.

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INTERRELATIONS OF THE RESPIRATORY CENTERS IN THE CAT¹

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In a previous study of the respiratory responses to stimulation of the brain stem of the cat with the Horsley-Clarke instrument (Pitts, Magoun and Ranson, 1939), special emphasis was placed on two types of response elicited from the medulla oblongata. These responses consisted of a tonic maximal inspiration and a tonic (and in some instances maximal) expiration, both of which could be maintained over considerable periods of time without interruption by rhythmic respiration. The following lines of evidence presented in that paper seemed to justify the conclusion that the 2 types of response mentioned above were elicited by stimulation of inspiratory and expiratory divisions of the medullary respiratory center. The inspiratory and expiratory responses are localized within a region of the reticular formation which has been vaguely defined as the respiratory center by previous workers in the field. These responses are coördinate respiratory acts involving both thorax and diaphragm. They are probably not due to stimulation of afferent pathways to or efferent pathways from the respiratory centers. They are invariable responses only quantitatively influenced by strength or frequency of stimulation. These responses are dominant over respiratory and vascular reflexes. They are independent of anesthesia. Coördinate respiration may be attained by rhythmic stimulation of these centers.

Further study of these centers seemed advisable in order to gain some insight into their mode of action in normal respiration. Accordingly we shall present in this paper results of investigation of 1, the effects on responses from the centers of excitation of respiratory afferents; 2, connections within the centers, and 3, the relation of these centers to apneustic respiration.

METHODS. We have used cats anesthetized lightly with nembutal (20 mgm. per kgm.), given intravenously, or decerebrated either intercollicularly or at a pontile level. Bipolar electrodes insulated except at the tips clamped in the Horsley-Clarke stereotaxic instrument were

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² Fellow of the Rockefeller Foundation.

oriented in the brain at the desired level. It was usually possible to place the tip of the electrode in the inspiratory or expiratory center as desired without preliminary exploration, though in order to improve the response it was often desirable to shift the electrode a millimeter or so. Stimulating current was obtained from a thyratron stimulator, calibrated oscillographically as to voltage and frequency with the electrodes in the brain. A constant frequency of 240 stimuli per second has been used in all experiments. Respiration was recorded by means of a spirometer built in the form of the familiar Krogh basal metabolism machine. Since the system is a closed one it was filled with oxygen and carbon dioxide was absorbed by a soda lime tube next to the tracheal cannula, so arranged as to reduce to a minimum dead space. The apparatus was refilled with oxygen at intervals determined by the metabolism of the animal. As was emphasized in the preceding paper this recording system is totally uninfluenced by extraneous motor activity of the animal, whereas abdominal or thoracic tambours were so affected by torsion of the animal as to be completely unreliable.

Excitation of respiratory afferents. It was apparent from results which were presented in a previous paper (Pitts, Magoun and Ranson, 1939) that stimulation of the inspiratory center must lead to maximal lung expansion and excite maximally those vagus afferent fibers sensitive to lung stretch. Since it is generally conceded that the play of vagus afferents on the respiratory center is capable of modifying its activity (Adrian, 1933) we explored the possibility that such afferent activity might alter responses to stimulation of the centers. Three types of experiments were repeatedly performed, 2 of which were consistently negative and will be only briefly described. The animal was inflated by about 25 cc. by placing a weight on the spirometer can. Reactions to both inspiratory and expiratory center stimulation (including minimal stimuli) were essentially the same as in the uninflated condition. Deflation by overcounterbalancing the spirometer was similarly without any observable effect though in each case the typical rate and amplitude changes in respiration were noted prior to stimulation. Similarly tonic effects on the inspiratory and expiratory centers by intact vagal innervation as compared to sectioned vagi could not be consistently demonstrated by comparing stimulation of the centers before and after vagal section. Occasionally it appeared on section of the vagi that the midposition of the chest shifted towards a more inspiratory position and thus inspiratory effects were slightly reduced while expiratory responses were somewhat increased. However, no consistent vagal effects could be demonstrated by the above mentioned experiments.

Central stimulation of the vagus by a Harvard inductorium (1.5 volts in the primary) in some cats yields clear-cut inspiratory reactions to weak stimulation (11 cm. coil setting) and expiratory reactions to stronger stimulation (7 cm.). It is evident from figure 1 that while the expiratory

hypertonus obtained on stimulation of the vagus (A) in some instances appears to be of similar magnitude to that obtained from stimulation of

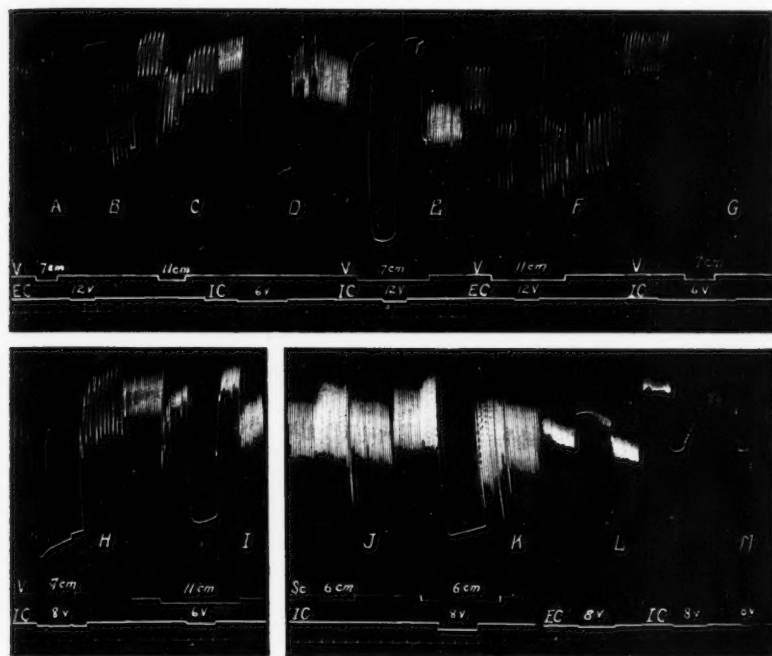


Fig. 1. Spirometer tracings of respiratory reactions resulting from stimulation of the medullary inspiratory and expiratory centers and afferent nerves. Inspiration down, expiration up in all records. Stimulation is indicated by the signal markers. Where voltages are indicated on the records stimulating current was obtained from thyatron controlled condenser discharges. Where coil distances in centimeters are given the stimulating current was obtained from an inductorium with 1.5 volts in the primary. V, central vagal stimulation; Sc, central sciatic stimulation; EC, expiratory center stimulation; IC, inspiratory center stimulation; time in all records 6 second intervals, except figure 3A to D, 3 second intervals.

A and C, reactions to strong and weak central vagal stimulation, compared with B and D, reactions to expiratory and inspiratory center stimulation. E, expiratory vagal reaction abolished by stimulation of the inspiratory center; F, inspiratory vagal reaction abolished by stimulation of the expiratory center. G, submaximal inspiratory center reaction reduced by strong central vagal stimulation; H, maximal inspiratory center stimulation unaffected by strong central vagal stimulation; I, inspiratory vagal response increased by stimulation of the inspiratory center. J, hyperpnea due to central sciatic stimulation, abolished by inspiratory center stimulation, K. L and M, expiratory and inspiratory center stimulation during panting resulting from overheating.

the expiratory center (B), the inspiratory hypertonus of vagal origin (C) in no way approaches that obtained on stimulation of the inspiratory center

(D). The expiratory effects of central vagal stimulation, however, may be completely abolished by stimulation of the inspiratory center with moderate stimulus strength (fig. 1E). Similarly the inspiratory effect of stimulation of the vagus may be abolished by stimulation of the expiratory center with a moderate stimulus strength, figure 1F. Thus stimulation of the appropriate center dominates the effect obtained from central vagal stimulation. However, if a submaximal inspiratory hypertonus is produced by stimulation of the inspiratory center, it may be reduced in magnitude by strong stimulation of the vagus, figure 1G, whereas maximal stimulation of the inspiratory center is unaffected by vagal stimulation, figure 1H. The inspiratory hypertonus produced by central vagal stimulation is readily increased by stimulation of the inspiratory center, figure 1I, whereas the converse cannot be demonstrated. If a reactive point within the expiratory center is stimulated maximally then no further increase in expiration is noted on strong stimulation of the vagus. In some cats an increase in expiration over that obtained on central vagal stimulation could be demonstrated on stimulation of the expiratory center but in these the vagal expirations were relatively poor.

The dominance of the inspiratory and expiratory effects of medullary center over vagal stimulation would argue for the thesis that we are stimulating the respiratory centers themselves for the vagus is a respiratory afferent par excellence. Our inability to show effects of tonic vagal activity or of stretch excitation of the lungs is easily explicable on the basis of this marked dominance of medullary center stimulation over central vagal stimulation. Another line of evidence is obtained from stimulation of the sciatic which leads to increased rate and amplitude, especially of the expiratory phase, figure 1J. The effect of stimulation of the inspiratory center is shown to be dominant over stimulation of this type of respiratory afferent, figure 1K. Another type of respiratory control is that descending from higher brain stem levels mediating polypneic panting in response to heat. Stimulation of the inspiratory and expiratory center is shown in figure 1L and M to be dominant over such respiratory connections.

Connections within the centers. In our previous work (Pitts, Magoun and Ranson, 1939) it was demonstrated that the inspiratory center has considerable extent in the lower medulla being distributed over a region of the reticular formation encompassing a volume of approximately 30 cu. mm. It has been further emphasized that the local area stimulated is of the order of magnitude of 1 to 3 cu. mm., since the advancement of the electrode less than 1 mm. in some regions is sufficient to reverse the type of response obtained. Since this is true using stimulating voltages as high as 20 volts, it is evidently even more true employing the relatively low voltage required to excite the inspiratory center, namely, 1-8 volts. By

stimulating one region on one side of the medulla occupying a volume of 1/10th to 1/30th of the inspiratory center we have obtained inspirations which appear to be maximal, i.e., inspirations amounting to 200 cc. in a cat whose tidal air was 25 cc. If the response is maximal there must be either rich synaptic interconnections between the cells of the respiratory center or possibly at the site of the lower motor neurons of the phrenic and thoracic cord regions.³

A more direct test of this thesis is obviously necessary. To this end we utilized a dual electrode carrier holding 2 pairs of stimulating electrodes separated by 4 mm. which could be placed into symmetrical regions of the inspiratory center 2 mm. to either side of the midline. Through 1 pair of electrodes we could stimulate the left side of the inspiratory center with the thyatron stimulator and through the other pair we could stimulate the right side with a Harvard inductorium. Various stimulating voltages were applied to the left side and the response was matched on the right by altering the coil setting of the inductorium. In figure 2A are shown results obtained by a stimulating voltage of 2 volts on the left and a coil setting of 12 cm. on the right. The close identity of response is evident. Combinations of the 2 lead to increased response, half again as great as the sum of the 2 responses singly. Thus there is evidence that some of the neurons between the 2 electrodes are subliminally stimulated by each of the 2 electrodes, while combined stimulation leads to their excitation. In figure 2B are shown results obtained by stimulating the left side with 12 volts (current spread still less than 1.0 mm.) and the right side with a coil setting of 9 cm. Here it is apparent that the response is maximal from one pair of electrodes, for no further increase in response occurs on stimulation with the second. Hence all those neurons capable of being stimulated by one electrode must be activated either directly or indirectly by the other. The exact extent of the inspiratory center which must be directly excited to lead to activity of the whole, though not specifically determined, must be rather small.

We next attempted to determine what effect excitation of the inspiratory center might have on activity occurring on stimulation of the expiratory center and vice versa. In order to accomplish this an electrode composed of 4 wires was utilized, one pair of wires being cut off some 2 mm. above the lower pair. This electrode was lowered into the medulla so that the upper 2 wires fell in the expiratory center, and the lower 2 fell in the inspiratory center. Some little exploration was necessary often to get good responses

³ We believe the crossing to be within the centers from the work of Porter (1894), who showed by hemisection of the cord that crossing occurred at the level of the phrenic nuclei only after section of the contralateral phrenic nerve. Also Rijlant (1937) showed that splitting the medulla in the midline leads to the establishment of independent respiratory rhythms on the two sides of the animal.

of each type, and it was usually impossible to so localize simultaneously maximal inspiratory and maximal expiratory responses. Figure 2C shows the response to expiratory stimulation, to inspiratory stimulation and to combinations of the two. Expiration here is passive; the same reactions were obtained, however, where a more active expiratory apnea resulted. It is evident that expiratory center stimulation during inspiratory center activation leads to a decrease in inspiration, and similarly inspiratory stimulation during expiratory activation leads to diminution

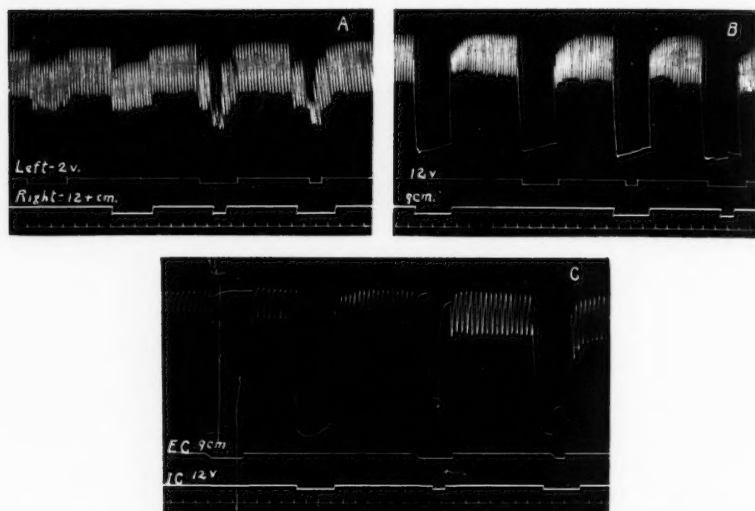


Fig. 2. Respiratory reactions to stimulation of the inspiratory and expiratory centers which indicate synaptic connections within and between the centers. In A and B two electrodes were placed in the inspiratory center 2 mm. to the right and left of the midline. A. note that weak stimulation of the 2 sides together produces an alteration of respiratory midposition half again as great as the sum of the 2 sides singly. In B no increase is noted from strong stimulation of the 2 sides together over that of the sides singly. In C a four pole electrode was placed in the medulla with the upper poles in the expiratory center and the lower poles in the inspiratory center. Simultaneous stimulation demonstrates mutual inhibition of the 2 centers.

in expiration. The dominant effect is usually obtained with inspiratory center stimulation. It is quite evident that stimulation of the inspiratory center must lead through synaptic interconnection not only to activation of many inspiratory neurons not directly affected by the stimulus, but also must produce an inhibitory effect on the expiratory center. Conversely, expiratory center stimulation probably in like manner spreads to expiratory neurons not directly excited as well as to the inspiratory center. The prominent effect of expiratory center stimulation, as emphasized previously (Pitts, Magoun and Ranson, 1939) is inhibition of inspiratory activity.

With the experimental methods at hand it is obviously possible to control the animal's respiration and to some degree its depth. It must be emphasized that the entire central respiratory mechanism is intact, the tendency always being for centrally controlled spontaneous respiration to appear except as this is dominated by excitatory and inhibitory effects of our stimulation. Figure 3 indicates a few of the ways in which respiration may be controlled. In figure 3A inspiration is produced by stimulation of the inspiratory center, expiration being spontaneous. Although the

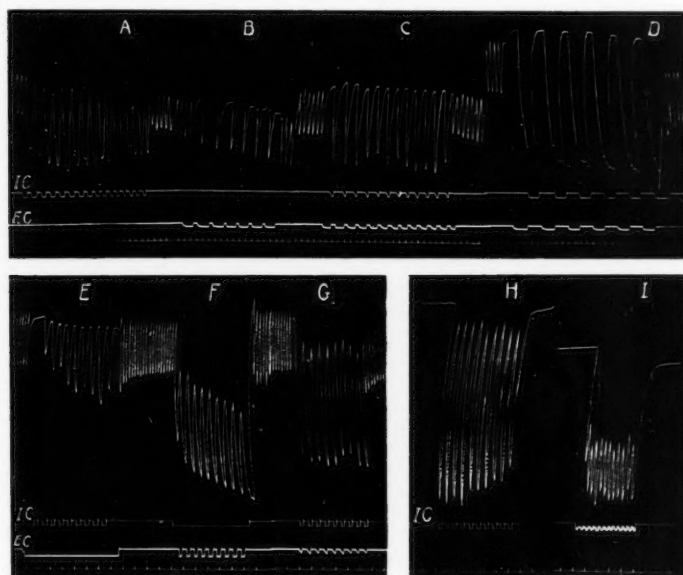


Fig. 3. Control of respiratory rhythm by rhythmic stimulation of the inspiratory and expiratory centers. In A the inspiratory, and in B the expiratory centers are rhythmically stimulated. In C, D and G the 2 centers are stimulated alternately. In E and F, one center is stimulated constantly while the other is stimulated rhythmically. In H and I the animal had ceased to breathe and respiration was induced by rhythmic stimulation of the inspiratory center.

inspiratory phase may be prolonged, since expiration is simultaneously inhibited, the spontaneous expiratory phase must be cut short, otherwise spontaneous inspiration will immediately follow. Figure 3B shows respiration in which stimulation of the expiratory center leads to expiratory apnea. Inspiration here is spontaneous. If on the other hand spontaneous respiration is inhibited then respiration may be driven more effectively. In figure 3E expiratory apnea is produced by maintained stimulation of the expiratory center and respiration is induced by rhythmic inspiratory center stimulation, and in figure 3F the converse is seen. In figure 3C,

D and G we have alternated the stimulation of the 2 centers at differing rates. Similar results may be obtained on inspiratory center stimulation in animals that have ceased to respire from shock, figure 3H. If in such an animal a maintained stimulus is applied to the inspiratory center and its intensity varied rhythmically, a maintained inspiratory hypertonus is obtained with superimposed respiratory movements, figure 3I.

Relation of centers to apneustic respiration. Though "inspiratory cramps" were first noted and described by Marekwald and Kronecker in 1880 the name "apneustic respiration" was coined by Lumsden in 1923 to describe these cramps, maintained for seconds to minutes, interrupted by a few gasps only to appear again. This type of respiration seen in pontile decerebrate cats was ineffective in ventilating the animals and death usually ensued after an interval. Lumsden ascribed the production of apneustic respiration to liberation of a medullary apneustic center from the rhythmic drive of a higher pneumotaxic center located in the uppermost pontile levels but he failed to note the important contribution of the vagus which Marekwald (1887) had established many years previously. Loewy (1888), Lewandowsky (1896), Henderson and Sweet (1929), Teregulow (1929) and Stella (1938) confirmed Marekwald in showing that apneustic respiration was produced only when the vagi were cut as well as the brain stem. Henderson and Sweet, however, described apneustic respiration as a postural decerebrate response of the chest having nothing to do with respiration, which postural mechanism could be held in check by intact vagal innervation. Stella (1938) concluded from his work on the variation of apneustic respiration with the carbon dioxide content of the blood supplying the medulla, that apneustic respiration being affected in the same direction as inspiratory activity in general, could be assigned to uncontrolled inspiratory activity when the vagi and upper brain stem levels were eliminated. Stella's method of reversibly cold blocking the vagi offered a ready means for close comparison of factors affecting rhythmic respiration and apneustic respiration, in addition to keeping the animals in a reactive state for a considerable period of time. To the end of correlating our results on the inspiratory and expiratory centers with this recent work on the apneustic center we utilized cats prepared essentially as described by Stella.⁴

Respiration in these animals was essentially normal after decerebration;

⁴ In our animals we approached the floor of the fourth ventricle from behind, beginning at the upper border of the foramen magnum and reflecting the occipital bone upwards to the tentorium. The cerebellar vermis was then removed with a blunt spatula and the floor of the fourth ventricle exposed to the level of the inferior colliculi. The section was made carefully with a bent pin, flattened and held in an artery clamp.

but in all, apneustic respiration was produced on section or cold blocking⁵ of the vagi (fig. 4A). With the vagus intact the inspiratory center was stimulated with a series of voltages from 1 to 12 volts, figure 4B. The vagus was then blocked and the inspiratory center stimulated again with these same strengths of stimulus, figure 4C. Recovery was permitted between each test. As may be seen the point stimulated in the inspiratory center was an especially reactive one, the effect becoming essentially maximal at 2-3 volts when respiration was normal (fig. 4B). Stimulation of the inspiratory center during apnea was effective in increasing the magnitude of the inspiration with even the lowest stimulus strength, 1 volt (fig. 4C). Furthermore, it is evident that the apnea persisting after the cessation of the stimulus is greater than the original; in other words stimulation of the inspiratory center has led to an increase in the apnea which increase outlasts the stimulus. As the voltage is increased to 3 volts there is increase in inspiratory activity which is maintained in part after cessation of the stimulus (fig. 4C). From 4 to 12 volts, however, the reaction changes. There is, as previously, an inspiratory addition on stimulation but with cessation of the stimulus there is complete and sudden disappearance of not only the increased inspiratory activity but of the apnea as well. Immediately after this complete inspiratory relaxation the apnea begins again to build up to its former level. Similar experiments were performed stimulating the expiratory center during the apneustic periods produced by blocking the vagi. Figure 4D shows reactions obtained on stimulating the expiratory center with 4, 8 and 12 volts. Similar reactions are shown in figure 4E during the apneas produced by cold blocking the vagi. It may be seen that 4 volts produced little if any effect, 8 volts caused relaxation which was maximal but poorly maintained whereas 12 volts caused expiration maintained over the 6 second interval of stimulation.

Additional ways of controlling respiration thus become available in the apneustic animal. The spontaneous apneustic inspiration may be rhythmically inhibited by stimulation of the expiratory center as shown in figure 4F. The spontaneous apneustic inspiration may be increased and released by stimulation of the inspiratory center with strong stimuli (8 volts), figure 4G. With weaker stimulation (4 volts) inspiratory excursions may be added onto the apnea only releasing the apnea at intervals (fig. 4G). Very weak stimulation (1-2 volts) increases the apnea which is maintained at a greater degree, the rhythmic stimulation of the inspiratory

⁵ Reversible cold blocking was accomplished with thermodes made from $\frac{1}{2}$ -inch copper rod, filed down, curved and smoothed at one end and embedded in bottles of cracked ice for a distance of 8 inches at the other end. Thermodes which are too cold soon injure the nerve.

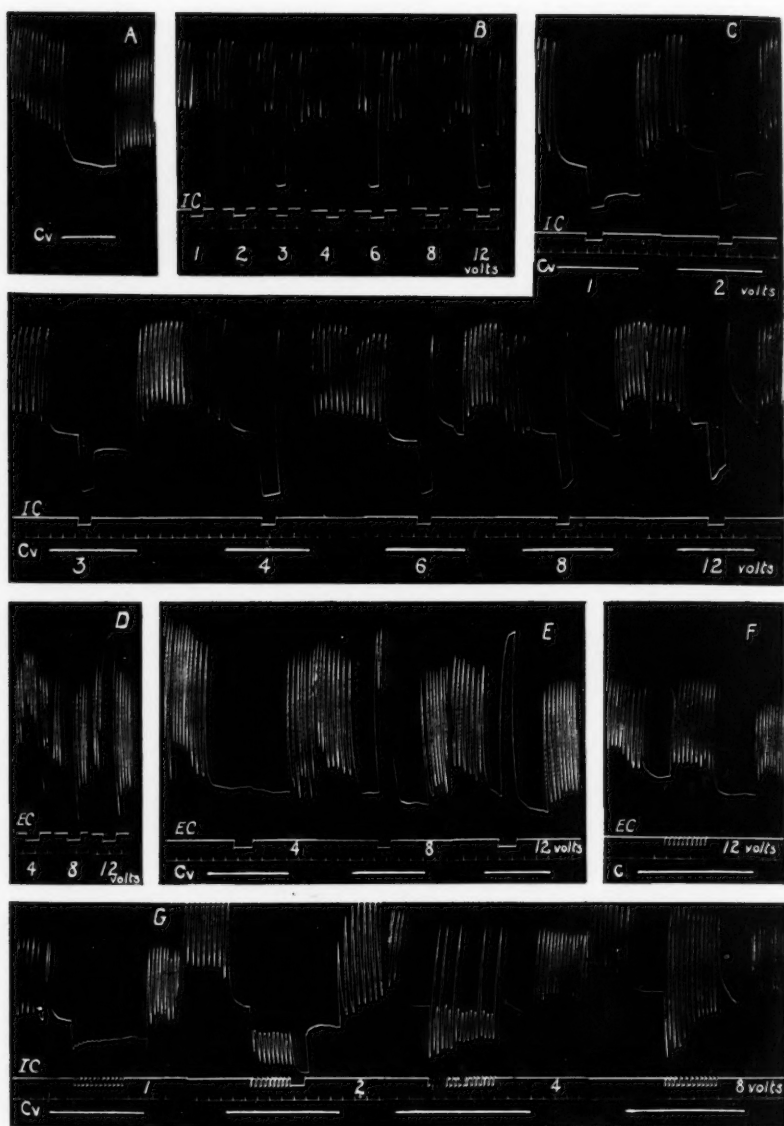


Fig. 4. Respiratory reaction from stimulation of the inspiratory and expiratory centers in pontile decerebrate animals during apneusis produced by cold blocking of the vagi. *Cv*, cold block applied to vagus. A, typical apneusis on cold blocking the vagi. B, inspiratory reactions to stimulation of the inspiratory center with voltages indicated. C, same voltages during apneusis produced by cold blocking the vagi. Note that from 1 to 3 volts the apneusis is maintained on cessation of the stimulus while from 4 to 12 volts it is temporarily released. D, expiratory reactions to stimulation of the expiratory center with voltages indicated. E, same voltages during apneusis. Note that 4 volts produced little effect, 8 volts expiration poorly maintained, while 12 volts maintains expiration. F, apneusis converted into rhythmic respiration by rhythmic stimulation of the expiratory center. G, apneusis converted into rhythmic respiration by stimulation of the inspiratory center.

center causing small respiratory excursions (2 volts) or only the faintest respiratory ripple (1 volt) to be superimposed (fig. 4).

In a few cats apneusis was produced by sectioning the vagi. This leads to the development of an initial prolonged apneusis as shown in figure 5A, following which the animal soon deteriorates and dies. However, some

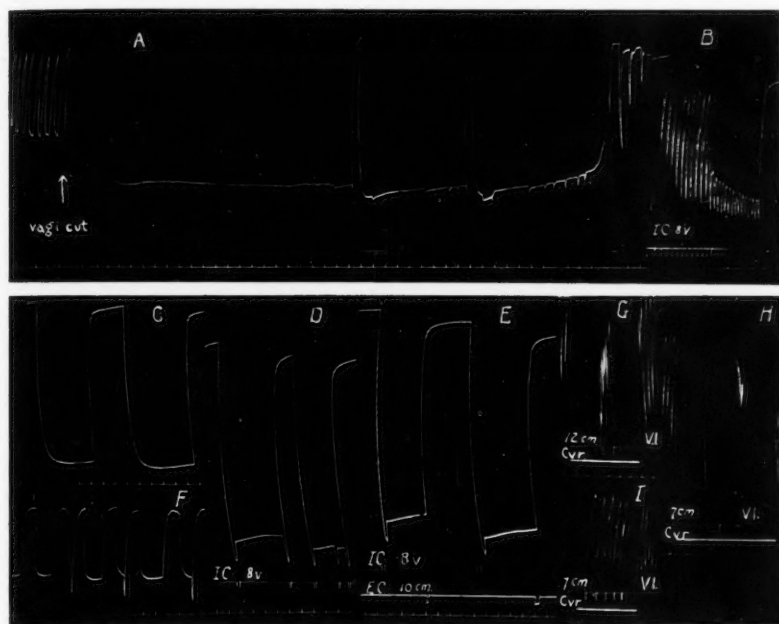


Fig. 5. Respiratory reactions from stimulation of the inspiratory and expiratory centers and the vagus in pontile decerebrate animals during apneusis produced by cutting or cold blocking the vagi. A, typical apneusis in a pontile decerebrate animal resulting from bilateral vagal section. B, stimulation of the inspiratory center after cessation of respiration in A. C, spontaneous apneustic respiration after prolonged artificial ventilation. D, stimulation of the inspiratory center in animal C. E, stimulation of the inspiratory and expiratory centers in a similarly prepared animal. F, spontaneous apneustic respiration with apneuses released by gasps. G, H, I stimulation of the central end of the sectioned left vagus during cold blocking of the right vagus.

cats after a period of artificial ventilation show a type of relatively rhythmic apneustic respiration, sufficient to maintain life when breathing oxygen from the spirometer (fig. 5C). In the expiratory apneic phase between the apneuses, stimulation of the inspiratory center causes the production of an apneusis which outlasts the stimulus, figure 5D. If again the inspiratory center is stimulated that same stimulus which produced the

apneusis is able to abolish it. By careful adjustment of voltage it is possible to produce apneusis by stimulation of the inspiratory center while only on repetitive stimulation is the apneusis abolished (cf. fig. 4G, 4 volts). Thus the release of apneusis by stimulating the inspiratory center depends on the voltage used, some change in the center brought out by repetitive stimulation and no doubt unrecognized fluctuations in the excitability of the center. In figure 5F is a record of spontaneous apneustic respiration in a cat with sectioned vagi which shows a spontaneous release of the apneuses due to a gasp. In those apneuses on which a gasp is superimposed, the apneusis is released. In figure 5B is presented the result of rhythmic stimulation of the inspiratory center of the animal whose deterioration is shown at the end of figure 5A. It is seen that though spontaneous apneustic inspiration is lost the inspiratory center is still excitable to rhythmic stimulation. Furthermore, with the ventilation provided there returns a gradually developing apneusis which at the end is released by that same inspiratory center stimulation which produced it.

It is possible to inhibit apneustic inspiration by faradization of the central end of one cut vagus during cold blocking of the other. Fairly regular respiration may be induced by such rhythmic vagal stimulation (fig. 5I). However, maintained weak central stimulation of the vagus leads to development of a spontaneous rhythmic respiration (fig. 5G) for the duration of the stimulus, while strong stimulation leads to apnea in expiration which far outlasts the duration of the stimulus and which is followed by a brief period of rhythmicity (fig. 5H).

The above experiments with apneustic respiration seem to us to be conclusive proof that the apneustic center of Lumsden is our inspiratory center, the activity of which is released from control of descending tracts from higher levels and from vagal afferent control. It is well to point out that Stella (1938), Barcroft (1934) and Marckwald (1887) concluded that apneusis results from unrestrained activity of the inspiratory center.

Discussion. In a preceding paper (Pitts, Magoun and Ranson, 1939) it was argued that the inspiration obtained by stimulation of the inspiratory center must be maximal (200 cc. in an animal whose normal tidal air is less than 25 cc.). Since the region locally stimulated by the electrodes is relatively small (less than 3 cu. mm.) whereas the extent of the inspiratory center is relatively large (over 30 cu. mm.), there must be extensive synaptic interconnection within the center such that excitation of a small part can lead to maximal response of the whole. Confirmation of this view has been obtained in the present paper by stimulation of symmetrical points within the center 2 mm. to either side of the midline. Stimulation with strong currents produces a maximal response from either pair of electrodes which is not increased when both are activated simultaneously. It has been possible with weak stimuli to show the existence of a sub-

liminally excited region between the 2 electrodes in which summation can be demonstrated.

It is evident from studies of Adrian and Bronk (1928) and Bronk and Ferguson (1935) that the degree of inspiratory activity of the diaphragm is proportional to the frequency of impulses over the phrenic nerves, while the degree of activity of the intercostal muscles is proportional to both the frequency in any one fiber and the number of active fibers. The results presented in the preceding paragraphs seem to indicate that variation in degree of inspiration on stimulation of the inspiratory center results from variation in numbers of active inspiratory center neurons. It is possible that integration at the level of phrenic and thoracic cord nuclei results in variation in impulse frequency in the phrenic nerve and impulse frequency and number of active units in the intercostal nerves.

In addition to spreading excitation within the inspiratory center it has been possible to demonstrate reciprocal inhibition of one of the centers from stimulation of the other. Thus stimulation of the expiratory center during inspiratory center stimulation leads to a reduction in inspiration. The converse is also true. We feel that this relationship is most easily explained by assuming that the inspiratory center neurons are not only richly interconnected, but through connections with the expiratory center are able to bring about an inhibition of its activity (cf. half center hypothesis of Brown, 1911, 1912). Similarly the expiratory center neurons are interconnected and are able to bring about inhibition of inspiration through connections with the inspiratory neurons. These relations are graphically shown in a schematized representation of respiratory linkages (fig. 6) which will be further developed in the succeeding pages.

The similarity between strong central vagal excitation and expiratory center stimulation in both normal and apneustic animals is close enough to lead to the belief that the vagal effects are mediated through the expiratory center. As was pointed out in our preceding paper, however, the anatomical extent of the expiratory center is not that of the entering vagal rootlets or their central connections in the nucleus of the tractus solitarius. As shown in figure 6, we believe that vagal effects are relayed through the nucleus of the tractus solitarius to the expiratory center where its excitation leads to expiration and simultaneous inhibition of inspiratory activity. Strong inspiratory center stimulation is, however, dominant over central vagal or even over expiratory center stimulation, a fact in line with the view generally held of the dominance of the inspiratory act in normal respiration.

Our results in line with Marekwald, Loewy, Lewandowsky, Henderson and Sweet and Stella have shown that in the absence of vagal innervation it is necessary for continuance of rhythmic respiration that the brain stem above the upper levels of the pons be in functional connection with

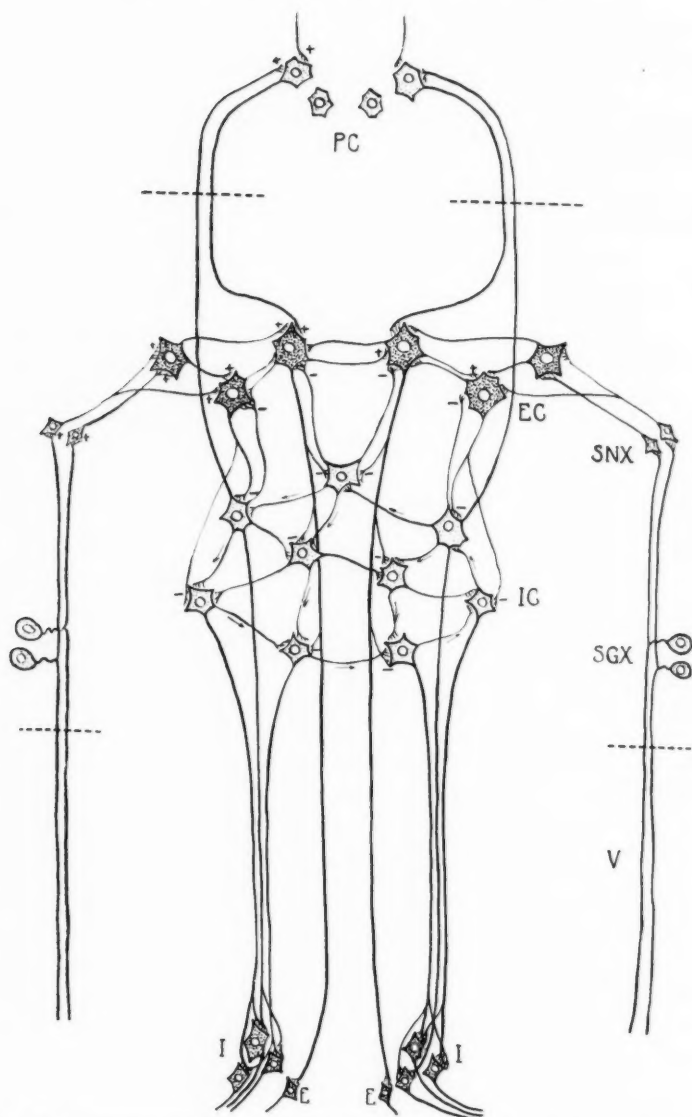


Fig. 6. Diagrammatic representation of possible respiratory linkages which would account for rhythmic respiration and apneusis. *E*, expiratory motor neurons in thoracic cord; *I*, inspiratory motor neurons in thoracic and cervical cord; *EC*, expiratory center; *IC*, inspiratory center; *PC*, "pneumotaxic" center; *V*, vagus; *SGX*, sensory ganglion of vagus; *SNX*, sensory nucleus of vagus. For explanation of figure, see text.

the inspiratory and expiratory centers of the medulla. In the absence of such connections (i.e., with pontile decerebration) and with the vagi blocked (see broken lines in fig. 6), there develops, as has been so admirably shown by Stella, a condition of tonic inspiration termed by those following Lumsden, "apneusis." Stella, by showing the similar reaction of normal rhythmic inspiration and apneusis to changes in carbon dioxide tension, has concluded that apneusis is unrestrained activity of the inspiratory center. We concur in this opinion and have added the pertinent facts that like inspiration, apneusis may be inhibited by stimulation of the expiratory center or by strong central stimulation of the vagus. An apneusis may be induced in an animal showing spontaneous rhythmic apneustic respiration by a brief stimulus applied to the inspiratory center. Weak or moderate stimulation of the inspiratory center during an apneusis increases the degree of apneusis which increase is maintained following cessation of the stimulus. Furthermore, we have shown that with strong stimulation of the inspiratory center that, although inspiration is increased markedly, in place of maintaining this increase on cessation of the stimulus there is immediate cessation of all inspiratory activity including the apneustic activity. After this sudden expiratory relaxation there occurs a building up of the apneusis to its original prestimulation level.

Release of apneusis after strong stimulation of the inspiratory center finds ready explanation in the silent period described in connection with reflex activity in the spinal cord. From a study of action potentials recorded from the flexor muscles of the foot during voluntary extension of the leg, it has been shown by Hoffman (1920) that if the peroneal nerve is stimulated 2 volleys of impulses may be recorded from the muscle, one due to direct excitation of the nerve, the other reflex. Following the second reflex volley there is a cessation of muscle action currents for a time which Gasser (Erlanger and Gasser, 1937) has shown approximates the duration of the period of positive after potential and decreased excitability in the cord resulting from the reflex volley. We are perhaps justified in assuming after strong stimulation of the inspiratory center excitability is so reduced as to momentarily block the flow of spontaneous impulses through the center with consequent release of the apneusis.

These results we feel can be synthesized into a logical, though admittedly hypothetical explanation of both apneusis and the action of the respiratory centers as a whole. With release of the inspiratory center from inhibitory activity of the vagus and from the pons (see broken lines in fig. 6), a stimulus originating in a few inspiratory neurons tends to spread throughout the inspiratory center through the rich synaptic connections of its constituent cells (gradual build-up of apneusis). It spreads likewise to the expiratory center where it causes inhibition of any expiratory activity. Then through various reëntry paths it tends to establish itself as a permanent

circus (maintenance of apneusis).⁶ This circus can scarcely include all the inspiratory neurons for weak or moderate stimulation of the inspiratory center can bring into activity new units (facilitation) which once in activity may remain in the circus after cessation of the stimulus (increased inspiration maintained as increased apneusis after cessation of stimulus). Stimulation of the expiratory center directly or indirectly through its connection with vagal afferents leads, as we have suggested previously, to inhibition of inspiration through raising of synaptic resistance between neurons of the inspiratory center (breaking the circus) as well as bringing about in some cases active expiratory activity. Such increase in synaptic resistance within the inspiratory center may merely be effective in eliminating some neurons from the circus (reduction in apneusis) or may abolish the circus (complete expiratory relaxation or active expiration). A strong stimulus, however, to the inspiratory center tends to set into activity simultaneously many neurons of the center producing maximal inspiration (replacing the circus with synchronous activity of many neurons). The depression of excitability in many neurons, following their synchronous activity, at the cessation of the stimulus interrupts and prevents reestablishment of the circus immediately (complete expiratory relaxation on cessation of a strong inspiratory stimulus). As recovery progresses the circus reestablishes itself (building up of apneusis again). With progressive apneusis and consequent cessation of pulmonary ventilation there occurs acute hypercapnia and oxygen lack which together may act to raise synaptic resistance within the inspiratory center leading to periodic failure of the circus (expiratory gasps) soon followed by another brief apneustic period and eventually death.

If these deductions concerning the mechanism of apneusis are correct interpretations of the experimental facts, we can make some further speculations as to the nature and mechanism of "pneumotaxis." We believe that there are 2 levels of similar "pneumotaxic" function, one vagal, the other upper pontine, either of which alone is capable of maintaining rhythmic respiration. On the action of the vagus some evidence has already been presented. We have shown that strong central stimulation of the vagus leads to expiration and to some degree of inhibition of the inspiratory activity produced by stimulation of the inspiratory center. Similarly central vagal stimulation inhibits apneusis. It is logical to assume that

⁶ Since the inspiratory center can be excited as a whole from an electrode placed anywhere within it, multiple intercellular connections must be present. Such a system involving links containing many neurons may lead to the establishment of a self-exciting reentry system as described by Ranson and Hinsey (1930, p. 491). While other explanations of the results are possible, e.g., a pacemaking group of neurons emitting a constant stream of impulses, or even asynchronous spontaneous discharge of many neurons, the present hypothesis seems to fit the experimental results on hand somewhat more adequately.

the spontaneous circus impulse spreading through the inspiratory center and leading to progressive increase in inspiration might lead to the setting up in the stretch vagal receptors of impulses which would bring about inspiratory inhibition (see Adrian, 1933), and thus rhythmic respiration whose rate and depth would be essentially determined by the synaptic resistance between cells of the inspiratory center (see fig. 6 for diagrammatic representation of the vagal reflex).

On the action of the upper pontine levels we have little direct evidence at present. The action of the upper pontine level may be pictured hypothetically as being essentially like that of the vagus. Thus this level might be progressively excited by successive activity in more and more units of the inspiratory center until its outflow to the expiratory center becomes great enough to lead to its excitation and thus to inspiratory inhibition.

Were this explanation of the action of the vagus and upper pontine levels correct, it is obvious that a real "pneumotaxic center" in the sense of a center driving respiration through spontaneous activity of its own is nonexistent. The action of the vagus is undoubtedly reflex. The possibility that a similar sort of activity is mediated by upper pontine levels as a result of ascending connections from the inspiratory and descending connections to the expiratory center is not a wholly unjustified assumption. The function of pneumotaxis would thus revert to the inspiratory center and these two systems, one involving vagal afferents, the other connections through the pontine pneumotaxic region. Rate as well as depth of respiration would become a function of the ease of spread of the inspiratory impulse throughout the center and the effectiveness of the connections through vagus and upper pontine levels in inhibiting the activity. These two latter factors could be influenced by local conditions (carbon dioxide, etc.) within the center itself as well as by impulses descending from higher levels or ascending from lower levels.

It is well to compare the foregoing hypothesis with that of Marckwald (1890). He believes that there are two respiratory centers, one inspiratory and one expiratory, the inspiratory center being the one of greatest importance. The act of inspiration sets up through the vagi and nuclei of the inferior quadrigeminal bodies impulses which are inhibitory to inspiration. Either can make up for loss of the other. If both are abolished inspiratory activity is unchecked and inspiratory cramps (apneuses) set in. If the trigeminal nucleus is intact it gradually assumes a tonic activity which eventually rhythmically inhibits the cramp and allows for some rather inadequate ventilation. It is evident that our hypothesis includes most of these points yet goes further into attempted explanation of the activity of the inspiratory center and the relation to it of the vagus and the expiratory center. We differ from Marckwald in assuming the correctness of Lumsden and others following him that the upper lying center for inhibition of inspiration lies not in the inferior colliculi but in the upper

pons. At present work on the location of this center and descending pathways is in progress.

Another more recent hypothesis is that of Barcroft (1934). He considers the gasp to be the fundamental respiratory act which is smoothed out into normal respiration under the influence of afferents impinging on the respiratory center. He feels that apneustic respiration results from partial deafferentation of the inspiratory center and expresses it as "inspiration which spends itself competing with inhibition" which inhibition though great enough to prevent the explosive burst of impulses producing the gasp, is not great enough to cut short inspiration and smooth it into the normal respiratory act. Thus the gasp explodes all the inspiratory energy at once, apneusis allows it to leak out over a long period of time and normal inspiration expends it in relatively small amounts, outflow being rhythmically inhibited. The essential difference in the three is in the degree of inhibition. This hypothesis we can subscribe to only in part. The gasp is no fundamental part of our system. We feel it is the final respiratory act of a deteriorating animal. We agree with Barcroft that there is probably minimal inhibition of the inspiratory center in the gasp and that the wave of excitation spreads throughout the center. But the spasmodic bursts of impulses so spreading can scarcely be transformed into an apneusis lasting up to several minutes by any sort of inhibitory control. We feel instead that the long lasting inspiratory activity of the apneusis is most adequately explained by some impulse circus or reëntry system in which excitation freed of inhibition is able to maintain itself until anoxia raises synaptic threshold to a point where the circus is broken (cf. fig. 5A). Only then do spasmodic gasps occur. We feel in the instance illustrated in figure 5 that the gasps may be likened to apneuses which fail to maintain themselves because of relatively high synaptic resistance and consequent failure to establish a circus rather than that they are apneuses released from inhibition.

CONCLUSIONS

The neurons of the inspiratory and expiratory divisions of the respiratory center are closely related synaptically. The relation is such that excitation of a small part of one of the centers may lead through synaptic interconnection to activity of the whole of that center. Activity in either center leads to inhibition of activity within the other.

The afferent fibers of the vagus affecting respiration are centrally connected with the inspiratory and expiratory centers probably through the nucleus of the tractus solitarius. Weak central vagal stimulation (circuit of low threshold) facilitates the spread of impulses through the inspiratory center. Strong central vagal stimulation (circuit of higher threshold) activates the expiratory center and consequently leads to inhibition of the inspiratory center. The latter circuit is probably the one by which im-

pulses arising from lung stretch bring about a cessation of inspiration and initiate expiration.

Apneusis results from maintained activity of the inspiratory center freed from the inhibitory influence of the above mentioned vagal lung stretch reflexes and from the influence of a "pneumotaxic" mechanism in the upper pons.

The similarities between apneusis and inspiration are as follows. Both inspiration and apneusis may be inhibited by stimulation of the expiratory center and also by strong central vagal stimulation. Stimulation of the inspiratory center in the expiratory interval of an animal showing rhythmic apneustic respiration induces an apneusis which far outlasts the stimulus. Weak stimulation of the inspiratory center during an apneusis increases the depth of inspiration, which increase outlasts the duration of the stimulus. Strong inspiratory center stimulation during an apneusis similarly increases inspiratory depth, but on cessation of the stimulus there is momentary release of the excess inspiratory and apneustic activity as well. This latter result is attributed to depressed excitability of connecting neurons within the center.

An hypothesis attempting an explanation of apneusis and normal respiration has been developed and discussed.

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ON THE EFFECT OF EPINEPHRINE ON BLOOD POTASSIUM

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Numerous observations (1, 2, 3, 4, 5) agree that epinephrine causes a prompt rise in serum potassium in the cat, rat, rabbit and dog. This effect is quite transient and is followed by a fall to a value below the pre-epinephrine level (3). In contrast to this, Keys (6) investigating the potassium response to epinephrine in man was unable to detect any early rise in plasma potassium and concluded that in man epinephrine effected only a decrease in plasma potassium. Since fundamental variations in reactions from species to species are rather rare further investigation of this apparent anomaly has been undertaken.

Two obvious differences exist between Keys' experiments on man and those which have been performed on animals. In general, arterial blood has been used for potassium analysis in animals whereas venous blood was used in man. Possible difference in the potassium level between arterial and venous blood samples drawn simultaneously at varying intervals following epinephrine has not been considered. Secondly, the ordinary procedure for demonstrating a rise in serum potassium following epinephrine involves rapid intravenous injection of the epinephrine and withdrawal of blood for analyses within 1 to 2 minutes thereafter. Keys' procedure was to inject the epinephrine over a period of $1\frac{1}{2}$ to 3 minutes and then draw blood $\frac{1}{2}$ to 3 minutes following the cessation of the injection. Flock et al. (7) have observed in the dog that continuous injection of epinephrine causes a decrease in serum potassium of from 19 to 32 per cent. This leads to the impression that possibly the rise in serum potassium occasioned by prolonged injection of epinephrine may not outlast that obtained by rapid injection of a single dose.

By the data to be presented we shall attempt to show that the confusion with respect to the effect of epinephrine on blood potassium is due to at least one of the factors outlined above and that when this is taken into account the apparently contradictory results may be readily harmonized.

METHODS. For the study of simultaneous arterio-venous serum potassium as well as the serum potassium-time curve following prolonged infusion of epinephrine, normal, adult, unanesthetized dogs were used.

The procedure in the experiments comparing arterial and venous serum potassium was to draw simultaneous control samples from the jugular vein and the femoral artery. One milliliter of 1:50,000 epinephrine was then injected rapidly into the jugular vein and blood samples drawn at 1 minute intervals for 3 minutes thereafter from both artery and vein. The serum potassium disappearance rate was determined by infusing epinephrine in dilution of 1:75,000 intravenously at the rate of 1 ml. per minute over a period of 10 minutes; venous samples being taken immediately before and at frequent intervals during the infusion.

The experiments of Keys were repeated in a fundamentally modified form using as subjects ambulatory, afebrile, hospital dispensary patients without arteriosclerotic changes or hypertension. No attempt was made to obtain basal conditions as the data are purely comparative. Blood samples were drawn from the median basilic vein before and after injection in the opposite vein of 0.1 ml. of epinephrine in dilution of 1:1,000. The injection of the epinephrine was made as rapidly as possible, that is, within a fraction of a second, the blood samples being taken within 30 to 90 seconds thereafter.

Potassium was determined in the serum by the method of Kramer and Tisdall (8).

RESULTS AND DISCUSSION. In figure 1, the relative arterio-venous serum potassium levels are shown following rapid intravenous injection of epinephrine. As can readily be seen, at the peak of the rise the serum potassium of arterial blood is notably greater than that of venous blood, then, with the onset of the fall the serum potassium level on arterial and venous sides begins to equalize so that by the time of the "after-fall" there is little difference between the two values. Clearly then, the very rapid loss of excess potassium from blood to tissues is a noteworthy fact and the investigator must anticipate a less marked rise in serum potassium following epinephrine if venous rather than arterial blood samples are taken for analysis.

In figure 2, the disappearance rate of potassium from the blood during constant infusion of epinephrine is shown. After the initial 2 minute peak the rate of fall is very rapid, reaching a level as much as 35 per cent below the control within 6 to 10 minutes. It is to be emphasized that the total duration of the serum potassium rise above control levels is only approximately $\frac{1}{2}$ minute longer than that following a single rapid injection of epinephrine. Accordingly, 3 to 4 minutes following epinephrine administration, whether by a single rapid injection or by infusion, there is very little chance of demonstrating an increase in serum potassium.

Another feature of figure 2 that must be mentioned is the initial fall noted in serum potassium when epinephrine is infused intravenously. This is noted 30 to 60 seconds after the beginning of infusion and although

slight was regularly observed and seems definitely significant. The possibility that a "pure" fall in serum potassium might be produced if the proper dosage and injection rate for epinephrine could be found immediately presented itself. To date, however, all attempts to do this in this laboratory have failed, the characteristic subsequent rise having always occurred in our experiments. At present we have no explanation for the phenomenon.

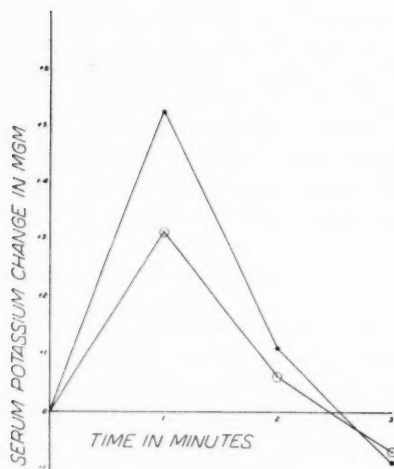


Fig. 1

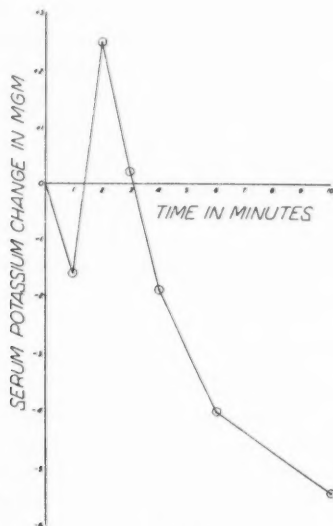


Fig. 2

Fig. 1. Ordinates show change in serum potassium in milligrams following rapid intravenous injection of 1 ml. of epinephrine in dilution of 1:50,000 in the dog. Abscissa records time in minutes after epinephrine injection. The curve is a composite one representing data from 6 experiments.

Fig. 2. Ordinates show change in serum potassium in milligrams during intravenous infusion of epinephrine in dilution of 1:75,000 at rate of 1 ml. per minute. Abscissa records time in minutes after beginning of epinephrine infusion. The curve is a composite one representing data from 4 experiments.

Since the potassium increase caused by epinephrine is due to its mobilization from the liver (3) it might be supposed that the ultimate decline in blood potassium during infusion of epinephrine is due to depletion of that source. To test this possibility we have in several cases at the 10 minute period, during a prolonged infusion of epinephrine, rapidly superimposed 1 ml. of epinephrine in dilution of 1:50,000. A blood sample was taken immediately before and 1 minute after the superimposed injection. Such samples showed in every case a significant increase in serum potassium

following the added epinephrine. The rise was, however, in general less marked than that which may be obtained from a previously untreated animal. This indicates either that the liver cell in some way adapts itself to a constant high level of epinephrine in the blood or that the rate of loss of potassium from blood to tissues is in excess of the rate of mobilization of potassium from the liver. The present data do not make possible a decision between these possibilities.

The technique used by Keys (6) is not adapted to demonstrate the exceedingly rapid rise and fall in the serum potassium level in man induced by epinephrine. It will be recalled that by the procedure used, something over two minutes at the very least, must have elapsed before the sample was taken. Apparently this is sufficient time for the "after-fall" phenomenon to have begun. The recorded data in the literature indicating the duration of the rise in potassium following epinephrine and the time of the beginning of the "after-fall" were not obtained on man and we see no

TABLE 1
Effect of epinephrine on serum potassium in man

| CASE NO. | CONTROL SERUM POTASSIUM | SERUM POTASSIUM 30-90 SECONDS AFTER EPINEPHRINE |
|----------|-------------------------|--|
| | mgm. % | mgm. % |
| 1 | 17.9 | 19.1 |
| 2 | 17.8 | 19.9 |
| 3 | 18.2 | 21.2 |
| 4 | 17.8 | 19.3 |
| 5 | 18.5 | 20.2 |
| 6 | 18.6 | 20.1 |
| 7 | 18.2 | 22.4 |

reason to suppose that the exact time relations of these responses should be identical from species to species.

It seems quite possible that the duration of the serum potassium rise in man, induced by epinephrine, is somewhat more ephemeral than in the experimental animal. Certainly slight rises in blood potassium would be detected with less ease in venous than in arterial blood.

From the data presented in table 1 we cannot but feel that intravenous injection of epinephrine results in a transient increase in serum potassium in man similar to that found in the experimental animal although the duration of the rise may be less. To demonstrate this very transient increase one must inject the epinephrine rapidly and draw blood samples within $1\frac{1}{2}$ minutes after the injection. In man as in the dog an interval of several minutes between injection of epinephrine and blood sampling allows sufficient time for the transient rise to have been replaced by a significant decrease in the blood potassium level.

CONCLUSIONS

1. The transient increase in potassium evoked by epinephrine injection is considerably less in venous than in arterial blood. A slight increase could pass unobserved if only venous blood was examined.

2. Infusion of epinephrine in the dog results in the blood potassium attaining a peak level within 2 minutes which is succeeded 1 to 2 minutes later by a comparable fall which may be as great as 35 per cent 10 minutes after beginning infusion.

3. Blood potassium concentration in man following epinephrine injection undergoes alterations identical in kind with those observed in the experimental animal.

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A STUDY OF THE COMPARATIVE TOXICITY OF CRYOLITE FLUORINE AND SODIUM FLUORIDE FOR THE RAT^{1,2}

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The increasing use of cryolite sprays as insecticides has raised the question as to the toxicity of these spray materials. Cryolite is a complex sodium aluminum fluoride having the formula $\text{AlF}_3 \cdot 3\text{NaF}$ and should be toxic in proportion to its sodium fluoride content. It contains 54.3 per cent fluorine. Smith and Leverton (8) have shown that fluorine contamination of a water supply in a concentration of one part per million causes mottled enamel in children. They calculated this to be equivalent to a fluorine intake of from 1 to 2 mgm. per child per day. Marcovitch, Shuey and Stanley (4) have pointed out that sprayed apples may contain nearly 1 mgm. of fluorine.

Marcovitch, Shuey and Stanley (4) have pointed out that the dangers of acute fluorine toxicity from cryolite are nil. They did not recognize the danger of possible chronic toxicity, which has been demonstrated to be of extreme importance in the mammalian. The first cases of chronic fluorine toxicity observed in man were described by Møller and Gudjonsen (6), and by Roholm (7). These occurred in cryolite factory workers who had been exposed to cryolite dust over long periods of time.

The researches presented here were an attempt to determine the relative toxicity of cryolite as compared to sodium fluoride.

EXPERIMENTAL. The toxicity of quite high levels of fluorine fed as cryolite was compared with that of the same levels given as sodium fluoride or as a mixture of sodium fluoride and aluminum chloride containing the same proportions of fluorine and aluminum as are present in a pure cryolite. Weanling albino rats weighing between 35 and 45 grams were used. The basal ration used in these studies had the following composition: yellow corn 55.75, wheat middlings 24.0, linseed oil meal 12.0, alfalfa meal 3.0, meat meal tankage 2.0, steamed bone meal 1.75, ground limestone 0.5,

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² Supported in part by a grant from the Works Progress Administration.

³ Moorman Manufacturing Company Fellow.

iodized salt 0.5, cod liver oil 1.0. Ten lots of five rats each were used. These were given the basal ration supplemented as follows:

| LOT | SUPPLEMENT TO BASAL RATION | THEORETICAL F CONTENT |
|------|---|--------------------------|
| | | <i>per cent</i> |
| I | 0.1105 per cent cryolite | 0.06 |
| II | 0.0554 per cent cryolite | 0.03 |
| III | 0.0276 per cent cryolite | 0.015 |
| IV | 0.132 per cent NaF | 0.06 |
| V | 0.066 per cent NaF | 0.03 |
| VI | 0.033 per cent NaF | 0.015 |
| VII | 0.0154 per cent NaF | 0.007 |
| VIII | 0.259 per cent $\text{AlCl}_3 + 6\text{NaF}$ | 0.06 |
| IX | 0.1295 per cent $\text{AlCl}_3 + 6\text{NaF}$ | 0.03 |
| X | 0.0648 per cent $\text{AlCl}_3 + 6\text{NaF}$ | 0.015 |

The basal ration contained 17.5 parts per million of fluorine. The cryolite used was added to the ration to supply 0.06, 0.03 and 0.015 per cent levels of fluorine, assuming the cryolite to be pure. Fluorine determinations by a modified Willard and Winter method showed that only 46.5 per cent of fluorine was present in the cryolite. This is 85 per cent of the fluorine theoretically present in pure cryolite. Dable and Wichmann (1) have shown that large amounts of aluminum interfere with the distillation of the fluorine. The following procedure was used to check the above results. A potassium carbonate fusion of the cryolite was made. The aluminum was precipitated from the dissolved melt with ammonium carbonate as described by Hillebrand and Lundell (3). Ammonia was removed by evaporation of the filtrate to a small volume. The resulting solution was neutralized to phenolphthalein with hydrochloric acid, made to volume, and aliquots were titrated with standard thorium nitrate solution. The fluorine content of the cryolite obtained by this method agreed well with the value obtained by direct distillation. The mixture of sodium fluoride and aluminum chloride which was fed contained 26.4 parts of NaF to 25.3 parts of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$. This supplied the fluorine and the aluminum in the same proportions as would be supplied by pure cryolite.

The rats were kept on experiment for twelve weeks. During this time body weight and feed consumption records were kept. The animals were sacrificed at the conclusion of the experiment, and the femurs and tibiae removed for fluorine analysis. The fluorine content of these bones was determined by distillation from perchloric acid and subsequent titration with thorium nitrate solution, as previously described (2).

A second experiment was set up to compare the skeletal storage of

fluorine resulting from the administration of small traces of fluorine. Three lots of weanling rats were fed the following ration: yellow corn 76, casein 15, yeast 3, cod liver oil 1, iodized salt 1, dicalcium phosphate 3.

Drinking water in a siphon water bottle attached to the cage was given to each group. The water consumption of each group was measured. The drinking water was given to the respective lots as follows: lot I, distilled water; lot II, distilled water containing 4 parts per million of fluorine as cryolite; and lot III, distilled water containing 4 parts per million of fluorine as sodium fluoride. At the end of eight weeks, the rats were sacrificed and fluorine determinations were made on the femurs and tibiae of each rat.

RESULTS. In the first experiment the 0.06 per cent level of fluorine caused an inhibition of growth in all cases (fig. 1). The growth inhibition

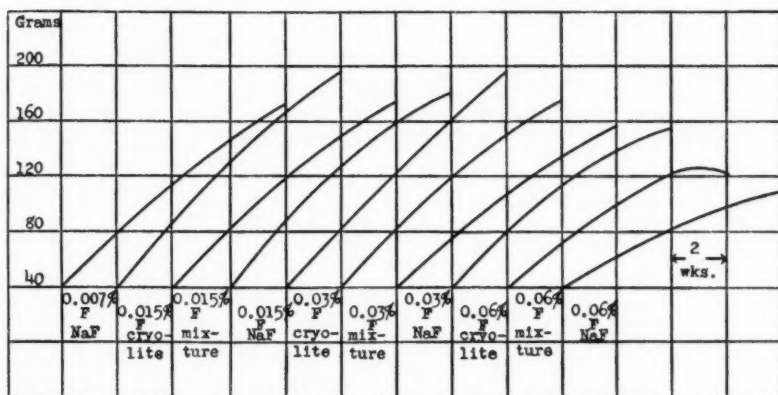


Fig. 1. Average growth of rats receiving various carriers of fluorine.

was much greater with sodium fluoride at this level than with the other fluorine carriers. In fact the average growth rate of the rats receiving 0.03 per cent fluorine as sodium fluoride was just about the same as for those receiving 0.06 per cent fluorine in the form of cryolite, or as the sodium fluoride-aluminum chloride mixture. Similarly a 0.015 per cent level of fluorine as sodium fluoride allowed a similar rate of growth to that allowed by a 0.03 per cent level of cryolite or of the sodium fluoride-aluminum chloride mixture. Normal growth occurred on the lower levels of fluorine regardless of the source. Thus the effects on growth indicate that cryolite and the sodium fluoride-aluminum chloride mixture are about one-half as toxic as sodium fluoride. In other words, the toxicity is directly proportional to the soluble fluoride present.

The condition of the incisor teeth is indicated in table 1. Bleaching

TABLE 1

The effect of various fluorine carriers upon the skeletal storage and the teeth of growing rats

| LOT | FLUORINE CARRIER | FLUORINE ADDED TO RATIONS | FLUORINE IN RATIONS | FLUORINE INTAKE | FLUORINE STORAGE IN SKELETON | CONDITION OF INCISORS* |
|------|--------------------------|---------------------------------|------------------------|--------------------|---------------------------------------|------------------------------|
| | | per cent | mgm./100 grams | mgm./kgm./ day | per cent | |
| I | Cryolite | 0.051 | 52.8 | 47.9 | 0.81 | +++ |
| II | Cryolite | 0.025 | 27.3 | 24.4 | 0.52 | +++ |
| III | Cryolite | 0.013 | 14.5 | 13.1 | 0.32 | ++ |
| IV | Sodium fluoride | 0.06 | 61.8 | 62.2 | 1.22 | ++++ |
| V | Sodium fluoride | 0.03 | 31.8 | 30.1 | 0.78 | +++ |
| VI | Sodium fluoride | 0.015 | 16.8 | 14.8 | 0.41 | +++ |
| VII | Sodium fluoride | 0.007 | 8.8 | 7.8 | 0.23 | + |
| VIII | 6NaF + AlCl ₃ | 0.06 | 61.8 | 58.4 | 0.95 | +++ |
| IX | 6NaF + AlCl ₃ | 0.03 | 31.8 | 33.6 | 0.67 | +++ |
| X | 6NaF + AlCl ₃ | 0.015 | 16.8 | 16.0 | 0.37 | ++ |

* Condition of incisors: +, incisors slightly discolored; ++, incisors mildly discolored for most part with some instances of chalkiness; +++, incisors bleached and elongated; +++++, incisors chalky, corroded and elongated.

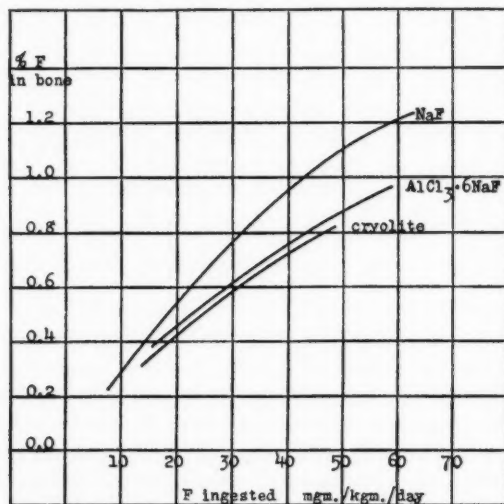


Fig. 2. Comparison of the fluorine storage in the bone with the fluorine intake of rats receiving various carriers of fluorine.

occurred at all levels of fluorine above 0.007 per cent. The 0.007 per cent level as sodium fluoride gave only slight discoloration. The degree of bleaching was parallel to the inhibition of growth.

Table 1 gives the fluorine intakes and the results of the analyses of the bones for fluorine. The actual fluorine contents of the rations supplemented with cryolite were lower than were those containing the other supplements, because the cryolite did not contain the theoretical amount of fluorine. Figure 2 gives a comparison of the fluorine storage as related to the fluorine intake for the three fluorine containing supplements. It is readily seen that the cryolite and the sodium fluoride-aluminum chloride mixture resulted in a similar ratio of fluorine stored to fluorine ingested. This ratio was less than that resulting from sodium fluoride. In this case sodium fluoride was not twice as toxic as was cryolite or the sodium fluor-

TABLE 2

Effect of fluorine in the drinking water upon the skeletal storage of fluorine in the rat

| FLUORINE INTAKE | FLUORINE CONTENT OF FEEDS (P.P.M.) | | |
|---|------------------------------------|---|---|
| | Lot I No added fluorine | Lot II 4 p.p.m. fluor- ine added as cryolite | Lot III 4 p.p.m. fluor- ine added as NaF |
| <i>mgm./kgm./day</i> | | | |
| Average fluorine intake from water..... | | 0.59 | 0.59 |
| Calculated total fluorine intake..... | 0.92 | 1.52 | 1.51 |
| | FEMUR AND TIBIA FLUORINE (P.P.M.) | | |
| | 581 | 724 | 692 |
| | 545 | 738 | 706 |
| | 564 | 688 | 711 |
| | 576 | 658 | 671 |
| | 552 | 648 | 696 |
| | 583 | 659 | 630 |
| | 571 | 626 | 650 |
| | 576 | 640 | 678 |
| | 559 | 680 | 669 |
| | 549 | 696 | 659 |
| Average..... | 566 | 675 | 676 |

ide-aluminum chloride mixture in causing a skeletal deposition of fluorine. This was true even at the higher levels of fluorine feeding. Cryolite and the sodium fluoride-aluminum chloride mixture caused almost the same fluorine deposition as did the sodium fluoride at the lower levels of fluorine administration.

When fluorine was supplemented at a level of 4 parts per million in the drinking water, no difference was observed in the skeletal fluorine as the result of giving either sodium fluoride or cryolite. This indicates that cryolite administered at these levels is equal in toxicity to sodium fluoride. Table 2 shows that a difference in intake of 0.6 mgm. per kgm. of body

weight per day of fluorine as cryolite or as sodium fluoride caused an increased skeletal storage of fluorine amounting to 110 parts per million. Considerable variation occurred between rats in each group. These variations seemed to be somewhat greater in the case of the rats receiving cryolite.

DISCUSSION. The similarities in the rates of growth of the rats receiving 0.03 per cent of fluorine as sodium fluoride and those receiving 0.06 per cent as cryolite or as the sodium fluoride-aluminum chloride mixture indicates that at these high levels of fluorine intake sodium fluoride is approximately twice as toxic as is cryolite. This does not hold for the lower levels. These results are in agreement with those of Smith and Leverton (8), who found that at a 0.0226 per cent level of fluorine, cryolite was distinctly less toxic than was sodium fluoride if growth and efficiency of utilization of the food consumed were used as criteria. They found that the same level of fluorine was required as sodium fluoride or as cryolite to give the first noticeable effect on the incisor teeth of rats. They found that ten times as much cryolite fluorine as sodium fluoride fluorine was required to inhibit growth. Our results do not agree with this, since we obtained the same rate of growth when fluorine was fed at a 0.03 per cent level as sodium fluoride and when it was fed at a 0.051 per cent level as cryolite. Smith and Leverton used a natural cryolite. We used a synthetic cryolite.⁴

Storage of fluorine in the skeleton, which is an exceedingly delicate measure of the effect of minute traces of fluorine, gave results which agreed with the observations on growth and on the incisors. The fluorine storage resulting from 0.03 per cent fluorine as sodium fluoride was not quite as great as that resulting from 0.051 per cent as cryolite. Thus at this level of fluorine intake cryolite was more than half as toxic as sodium fluoride. At the lower levels of intake there was less difference in the fluorine storage caused by the three supplements. At the low level of 4 parts per million of fluorine in the drinking water the storage from the cryolite was identical to that from sodium fluoride. These results show that at very low levels of fluorine intake, cryolite and sodium fluoride caused the same fluorine deposition in the skeleton. Marcovitch and Stanley (5) showed 4 parts per million of fluorine as cryolite in the solid ration to cause less fluorine storage than the same level as sodium fluoride in the drinking water. Figure 2 shows that the proportion of the ingested fluorine that was stored increased as the fluorine intake decreased. This increase was most marked in the case of cryolite and of the sodium fluoride-aluminum chloride mixture.

⁴ This was Aloreo Cryolite which was kindly supplied by the Stauffer Chemical Company.

Solubility appears to be a factor in the determination of the toxicity of fluorine compounds, especially at higher levels of fluorine intake. This factor undoubtedly accounts for the results obtained by Marcovitch, Shuey and Stanley (4). They fed cryolite to a rat in the proportion of 13,500 mgm. per kgm. of body weight during a period of 20 hours without fatality.

Our results show that even at very low levels of cryolite feeding, enough fluorine is absorbed to produce definite chronic toxicity. Fluorine has been shown to be just as well absorbed from cryolite as from sodium fluoride when given in minute amounts. These data indicate that minute amounts of ingested cryolite will prove as toxic as the same quantity of fluorine obtained from sodium fluoride.

SUMMARY AND CONCLUSIONS

Four parts of fluorine per million added to the drinking water as sodium fluoride or as cryolite resulted in identical storage of fluorine in the bones of the growing rat. At the higher levels less fluorine was found in the skeleton when it was fed as cryolite or as the sodium fluoride-aluminum chloride mixture than when it was fed as straight sodium fluoride. When fluorine was fed at a level of 0.06 per cent of the diet, sodium fluoride was approximately twice as toxic as was cryolite or the sodium fluoride-aluminum chloride mixture. Cryolite and the sodium fluoride-aluminum chloride mixture paralleled each other in toxicity.

These results lead to the conclusion that cryolite is toxic for the rat, that its toxicity is equal to sodium fluoride in trace levels, and at higher levels its toxicity is approximately half that of sodium fluoride. Further, evidence is presented to show that the toxicity of cryolite is roughly proportional to its soluble fluoride content. It seems that the NaF portion of the $\text{AlF}_3 \cdot 3\text{NaF}$ molecule is responsible for its toxic properties.

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ELECTROCARDIOGRAPHIC CHANGES AND CONCENTRATION OF MAGNESIUM IN SERUM FOLLOWING INTRA-VEINUS INJECTION OF MAGNESIUM SALTS¹

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In this study the electrocardiographic changes appearing during the intravenous injection of magnesium are compared with the associated changes in the concentration of magnesium in serum. Similar studies of calcium and potassium have previously been reported (4, 8).

METHODS. Seventeen dogs under local procaine anesthesia only were used. In eleven experiments an isotonic solution of magnesium sulphate was injected into the femoral vein at a slow uniform rate, while at intervals during the injection samples of blood were obtained from the jugular vein or from the heart for analysis of the concentration of magnesium in the serum. Serial electrocardiograms from lead II were also taken at intervals. The concentration of magnesium in the serum rose at an approximately uniform rate, so that the concentration corresponding to each electrocardiographic change could be obtained by interpolation between determined values. Whenever failure of natural respiration due to the effects of magnesium on the neuromuscular system occurred, artificial respiration was substituted without interrupting the injection, which was then continued until the death of the animal. Magnesium was determined by a slight modification of the method of Hald (2).

In six experiments magnesium chloride and calcium chloride were injected simultaneously, the technique being otherwise identical with that just described. The calcium of serum was determined by direct precipitation from serum as the oxalate, washing by centrifugation, and titration with potassium permanganate.

Five supplementary experiments were carried out using cats, four decerebrate and one under amytal anesthesia. With the exception of

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the anesthesia the procedure was identical with that described above in the dog experiments.

TABLE 1

Experiments with the injection of magnesium sulfate (0.154 M) intravenously into dogs. No other salts administered simultaneously. Local anesthesia only

| EXPERIMENT | WEIGHT OF DOG | AMOUNT OF SOLUTION INJECTED | DURATION OF INJECTION | RATE OF INJECTION | CONCENTRATION OF Mg IN SERUM | | | | REMARKS |
|------------|---------------|-----------------------------|-----------------------|-------------------|---|---------------|-------------------------------------|---------------------------------|---|
| | | | | | Time sample taken after injection | Concentration | Concentration at respiratory arrest | Concentration at cardiac arrest | |
| | kgm. | cc. | min. | cc./kgm./min. | min. | m. Eq./lit. | m. Eq./lit. | m. Eq./lit. | |
| 1 | 11.1 | 160 | 11.3 | 1.27 | 0 | 1.2 | 23.0 | 36.0 | |
| | | | | | 12 | 36.0 | | | |
| 2 | 11.9 | 340 | 24.0 | 1.19 | 0 | 1.5 | | | Animal survived |
| | | | | | 15 | 6.1 | | | |
| | | | | | 18 | 11.2 | | | |
| | | | | | 48 | 17.2 | | | |
| 3 | 8.6 | 110 | 12.0 | 1.44 | 0 | 1.1 | 17.5 | 17.5 | Salivation at 1.5 min. Heart widely dilated at post mortem; responded to mechanical stimulation |
| | | | | | 10.5 | 16.6 | | | |
| 4 | 8.5 | | 14.0 | | 0 | | 18.0 | 36.9 | Heart arrested at post mortem; soon started to beat at increasing rate for a short time |
| | | | | | 6 | 22.6 | | | |
| | | | | | 14 | 36.9 | | | |
| 5 | 8.0 | 118 | 11.0 | 1.34 | 0 | 1.5 | 17.0 | 37.8 | Ventricular fibrillation |
| | | | | | 11 | 37.8 | | | |
| 6 | 8.6 | (a) 57 (b) 45 | 10.0 4.7 | 1.01 1.12 | 0 | 1.7 | (a) 25.0 (b) 16.0 | (a) 27.3 (b) 39.2 | (a) First injection (b) Second injection |
| | | | | | 8 | 23.8 | | | |
| | | | | | 10 | 27.3 | | | |
| | | | | | Injection stopped for 12 min., then resumed | | | | |
| | | | | | 22 | 16.0 | | | |
| | | | | | 26.7 | 39.2 | | | |
| 7 | 13.0 | 80 | 3.5 | 1.76 | 0 | 1.4 | 27.8 | 27.8 | |
| | | | | | 3.5 | 27.8 | | | |
| 8 | 10.4 | 80 | 5.6 | 1.37 | 0 | 1.8 | 23.0 | 30.8 | |
| | | | | | 5.6 | 30.8 | | | |
| 9 | 16.1 | 220 | 14.8 | 0.92 | 0 | 1.9 | 19.0 | 36.3 (23.0) | Heart arrested at 36.3 m.Eq. Mg per liter but began again and continued for 14 min. with death at 23.0 m.Eq. Mg per liter |
| | | | | | 8.5 | 22.2 | | | |
| | | | | | 14.8 | 36.3 | | | |
| | | | | | Injection stopped | | | | |
| | | | | | 29.0 | 23.0 | | | |
| 10 | 7.4 | 175 | 16.8 | 1.41 | 0 | 1.7 | 19.0 | 44.2 | Vomited at 1.8 min. |
| | | | | | 9.5 | 28.2 | | | |
| | | | | | 16.8 | 44.2 | | | |
| 11 | 13.3 | 340 | 34.0 | 0.75 | 0 | 1.9 | 20.0 | 66.9 | |
| | | | | | 6.5 | 22.7 | | | |
| | | | | | 34 | 66.9 | | | |

RESULTS. I. *Experiments with the injection of magnesium sulphate alone in dogs.* A. *General toxicity.* In table 1 are summarized the protocols of ten experiments which were continued until death due to cardiac

arrest and of one in which the animal survived. In some experiments, i.e., nos. 3, 6 and 7, respiratory and cardiac arrest occurred simultaneously; in the majority, however, respiratory arrest appeared first, and after artificial respiration had been established, the heart continued to beat until a considerably higher concentration of magnesium in serum had been reached. In no case did cardiac arrest precede respiratory failure. The concentration of magnesium at which respiratory failure occurred ranged from 17 to 28 mEq. per liter, the higher values being associated with the more rapid rates of injection. With the exception of experiments 3 and 11, the concentration of magnesium associated with cardiac arrest fell between 27 and 44 mEq. per liter. The very high value (66 mEq. per liter) in experiment 11 was associated with a very slow rate of injection.

In only one experiment (no. 5) was ventricular fibrillation observed and here it may have been a postmortem development initiated by the needle puncture to obtain blood. In all others the terminal event was cardiac arrest. On opening the thorax after arrest it was usually possible to elicit a few regular beats by mechanical stimulation of the heart. The reversibility, in part at least, of the process of arrest is indicated by the sequence of events in experiment 6. Here arrest took place and the injection was stopped at 27.3 mEq. per liter; the heart then spontaneously resumed its beating. After some minutes a second injection was begun which was continued until a concentration of 39 mEq. per liter was reached before arrest again occurred.

B. Electrocardiographic effects. These manifest themselves chiefly in the heart rate and in conduction. (i) *Heart rate.* In figure 1 the mean rates for all experiments included in table 1 are plotted against the levels of magnesium in serum. There is an immediate sharp increase in rate, appearing before a 5 mEq. per liter level is attained. After this initial rise the rate gradually declines to its original level or somewhat lower as the concentration of magnesium continues to rise. Final arrest appears as a sudden cessation without further decline in the rate. This sequence of changes in the pulse rate in individual experiments is well illustrated in figures 2 and 3. (ii) *Changes in conduction.* In all experiments there is slowing in A-V conduction, as measured by a progressive increase in the P-R interval (figs. 2 and 3). In figure 1 the average P-R intervals for all experiments are plotted against the concentration of magnesium in serum. After a brief initial decrease the interval gradually lengthens, reaching as much as 0.30 second (fig. 3). Various grades of A-V block appeared in certain experiments (fig. 2). Sino-auricular block of 2:1 grade appeared in two instances, indicating that in this part of the conduction system also there may be depression of conductivity.

Some widening of the QRS complexes, indicative of intraventricular block, is present in most of the experiments. Starting with a normal

value of 0.4 second the width frequently doubled by the time of respiratory failure (figs. 2 and 3). In only a few instances was there further widening by the time cardiac arrest supervened. In some experiments only a slight widening of the QRS complexes occurred.

(iii) *Ectopic rhythms.* The single instance of fibrillation (expt. 5) has already been mentioned. It is difficult to ascribe this and certain other ectopic rhythms occasionally observed to the action of the magnesium, since they in general appeared immediately following a cardiac puncture. On the other hand, it is certainly clear that high concentrations of magnesium do not serve to inhibit the development of such arrhythmias. Thus

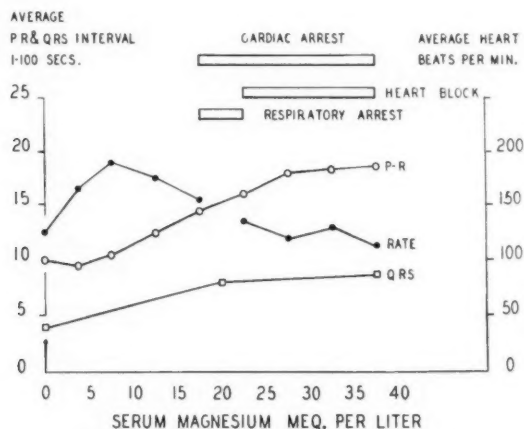


Fig. 1. Graph summarizing results of all the experiments of table 1. Average P-R and QRS intervals and heart rates are plotted against concentration of magnesium in the serum. In the upper part of the figure are indicated schematically the range of concentrations of magnesium at which respiratory arrest, heart block of various types, and cardiac standstill usually occurred.

in experiment 9 the cardiac puncture at the moment of respiratory failure produced a run of ventricular extrasystoles from multiple foci and a ventricular tachycardia reaching nearly 500 beats per minute. The magnesium content of the serum during the time of this development had attained a high value of 36 mEq. per liter. In experiment 4, in which the concentration of magnesium had reached a similar level, ectopic ventricular beats were recorded immediately before final arrest (fig. 2).

(iv) *T-wave changes.* Characteristic changes in the ST segment and in the T-wave were not observed. While inversion and changes in the amplitude of the T-wave were sometimes recorded (figs. 2 and 3), these changes were entirely lacking in some experiments and minimal in most.

II. *Experiments with simultaneous injection of magnesium and calcium salts in dogs.* In view of the known inhibition of certain of the effects

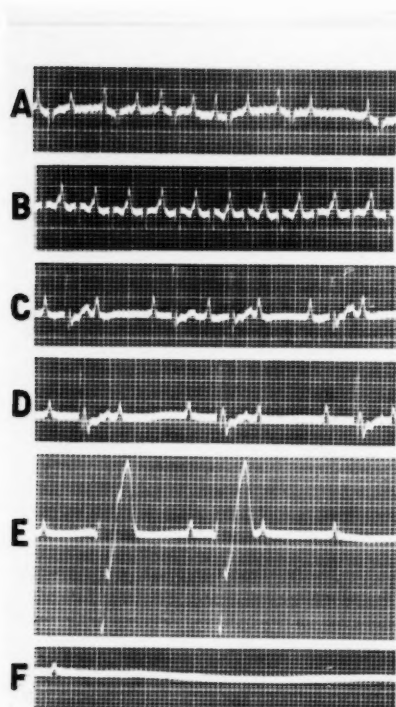


Fig. 2

Fig. 2. Electrocardiograms from experiment 4 of table 1 (dog). (A) Control. (B) 3 minutes after magnesium injection had begun; initial tachycardia. (C) 4 minutes. Slowing, 2:1 A-V block and changes in T-wave. (D) 11 minutes. 2:1 A-V block, P-R interval 0.26 second. (E) 12 minutes. Total block with ventricular beats. (F) Arrest.

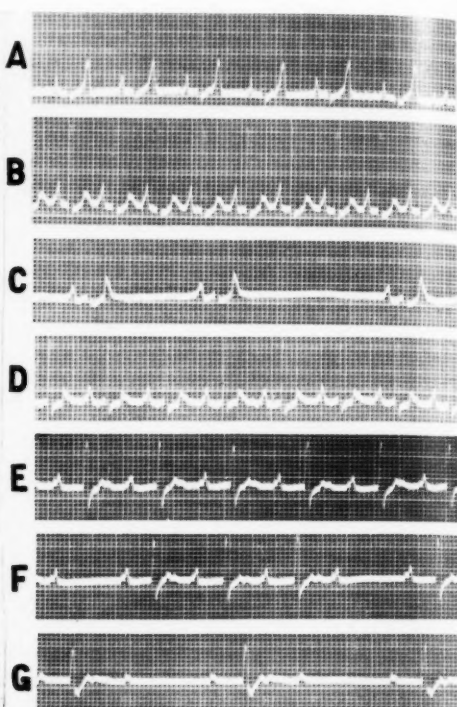


Fig. 3

Fig. 3. Electrocardiograms from experiment 11 of table 1 (dog). (A) Control. (B) 2 minutes after onset of injection. (C) 6.5 minutes. Beginning respiratory failure. (D) 12 minutes. Artificial respiration. (E, F and G) 18, 19, and 24 minutes respectively. This sequence shows changes in the heart rate, P-R and QRS intervals, and in the T-wave. P-R lengthening greater (0.30 sec.) in this than in any other experiment.

of magnesium on the nervous system by the injection of calcium salts (1), six experiments were carried out in which calcium chloride and magnesium chloride were injected simultaneously in varying proportions. The

protocols are summarized in table 2. In every experiment except no. 13 death occurred at a serum magnesium level at which death might be expected to follow the injection of magnesium alone (see table 1). In experiment 13 death was due to sudden ventricular fibrillation at a level of magnesium of only 14 mEq. per liter. However, the simultaneous

TABLE 2

Experiments with the simultaneous injection of magnesium chloride and of calcium chloride into dogs

Local anesthesia except as otherwise noted

| EXPERIMENT | WEIGHT OF DOG | COMP. OF SOLUTION | | AMOUNT OF SOLUTION INJECTED | DURATION OF INJECTION | RATE OF INJECTION | | CONCENTRATION OF Mg and Ca IN SERUM | | | | | | | REMARKS |
|------------|---------------|-------------------|-------|-----------------------------|-----------------------|-------------------|------|-------------------------------------|-----------------------------|------------------------------|-----------------------------|---------------------------------|------|---|---------|
| | | Mg | Ca | | | Mg | Ca | Time sample taken after injection | Concentration | | Conc. at resp. arrest Mg | Concentration at cardiac arrest | | | |
| | | | | | | | | | Mg | Ca | | Mg | Ca | | |
| | | | | | | | | | | | | | | | |
| kg. | m. Eq./lit. | m. Eq./lit. | cc. | min. | m. Eq./kg./min. | mg./kg./min. | min. | m. Eq./lit. | mg./100 cc. | m. Eq./lit. | m. Eq./lit. | mg./100 cc. | | | |
| 12 | 23.1 | 72.0 | 148.0 | 673 | 15 | 0.140 | 2.88 | 0 15 | 2.4 18.8 | 11.4 39.3 | 18.8 | 18.8 | 39.3 | Ether and nembutal supplementary | |
| 13 | 13.5 | 72.0 | 148.0 | 468 | 17 | 0.147 | 3.02 | 0 17 | 2.1 14.1 | 11.4 35.5 | | 14.1 | 35.5 | Death by fibrillation. Respiration continued for a short time | |
| 14 | 12.8 | 184.0 | 46.0 | 370 | 14 | 0.380 | 0.95 | 0 10 14 | 1.8 28.8 37.5 | 11.8 16.4 17.2 | 15.5 | 37.5 | 17.2 | | |
| 15 | 6.1 | 184.0 | 46.0 | 350 | 27 | 0.391 | 0.98 | 0 14.5 21.0 27.0 | 2.1 30.9 41.2 56.2 | 11.1 14.8 17.2 20.3 | 14.0 | 56.2 | 20.3 | Vomited at 3 min.; water not freshly distilled. Terminal ventricular fibrillation | |
| 16 | 17.7 | 176.6 | 58.8 | 874 | 45 | 0.194 | 0.65 | 0 23 45 | 1.5 21.8 34.4 | 11.9 19.0 21.1 | † | 34.4 | 21.1 | | |
| 17 | 11.1 | 176.6 | 58.8 | 368 | 20 | 0.293 | 0.97 | 0 10 20 | 1.6 28.6 32.9 | 11.8 20.3 20.3 | * | 32.9 | 20.3 | Vomited at 2.5 min.; freshly distilled water used | |

* Slow at 30.0. Not complete arrest.

† Slow at 21.0. Not complete arrest.

calcium concentration was 35.5 mgm. per cent, a concentration at which calcium alone may cause death from fibrillation (4). This single exception therefore is probably due primarily to calcium. The electrocardiographic records showed a mixture of such effects as might be due to calcium alone and those which might be due to magnesium alone.

Experiment 15 also terminated in ventricular fibrillation, appearing

after a brief interval of arrest. The calcium concentration at this time was 20 mgm. per cent, the magnesium concentration 56 mEq. per liter. This fibrillation is probably a postmortem effect, and so is to be classed with the instances of fibrillation occasionally observed after magnesium injection alone. In view of the relatively low concentration of calcium, it probably is not a simple calcium effect. This experiment demonstrates again that a high concentration of magnesium will not prevent the development of an ectopic rhythm.

In experiments 12 and 13 the calcium effects were the more prominent, in the remaining four experiments the magnesium effects predominated. This is in accord with the relative degree of increase of the concentration of the two ions in the respective experiments. Certainly there is no evidence of a protective action of calcium against the toxic influence of

TABLE 3
Summary of experiments with cats

| EXPERIMENT | PRELIMINARY PREPARATION | WEIGHT OF CAT | AMOUNT OF 0.154 M Mg SO ₄ INJECTED | DURATION OF INJECTION | RATE OF INJECTION | CONCENTRATION OF Mg IN SERUM AT CARDIAC ARREST |
|------------|-------------------------|---------------|---|-----------------------|-------------------|--|
| | | kgm. | cc. | min. | cc./kgm./min. | m.Eq./lit. |
| A | Decerebrate | 3.5 | 32 | 9 | 1.02 | 45.8 |
| B | Decerebrate | 2.5 | 34 | 21 | 0.65 | 60.5 |
| C | Decerebrate | 2.4 | 72 | 51 | 0.59 | 52.2 |
| D | Decerebrate | 2.3 | 43 | 38 | 0.49 | 52.8 |
| E | Sodium amytal | 2.0 | 52 | 27 | 0.98 | 44.0 |

magnesium on the heart. It is not possible from these few experiments to ascertain whether they may not be partially additive in their toxicity.

In all six experiments the increase in the P-R interval, so characteristic of magnesium effect, is present to its usual extent.

III. Experiments with the injection of magnesium salts in cats. The experiments with cats are summarized in table 3. The concentration of magnesium in the serum obtained from the heart at death is usually somewhat higher than that commonly found in dogs (see table 1). Part of this may be an artefact, since in smaller animals such as cats it is difficult to obtain blood from the heart without also drawing up some solution from the femoral vein receiving injection. This error must, however, have been small, and it is possible that these higher concentrations represent a true species difference. It should be remembered that the difference in anesthetic preparation makes these experiments not truly comparable with those on dogs. Nevertheless, with one exception, the sequence and character of the electrocardiographic changes were identical with those

in dogs (figs. 4 and 5). This exception is a complete absence of the initial tachycardia. The exception is probably correlated with the decerebrate

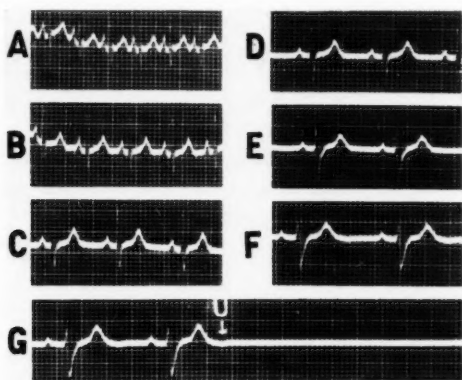


Fig. 4. Electrocardiograms from experiment (E) of table 3. (Cat). (A) Control. (B) 3 minutes after magnesium injection had begun. (C, D, E, F and G) 8, 13, 23, 26 and 27 minutes respectively. The last two complexes before arrest appear in (G). In (F) and (G) negative U waves are seen.

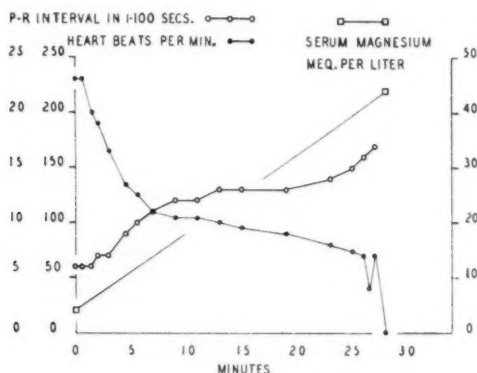


Fig. 5. Graph from the same experiment (E) of table 3. (Cat), showing the relation between heart rate, P-R interval, and concentration of magnesium in serum in a single typical experiment.

or anesthetized preparation of these cats rather than with the difference in species.

IV. Appearance of vomiting. One purely incidental observation is the occasional occurrence of vomiting during the course of injection of mag-

nesium into dogs, once in the magnesium series and twice in the combined magnesium and calcium series. Since no such effect was ever observed in experiments in which calcium alone was injected (4), it is reasonable to conclude that the magnesium ion is responsible for the vomiting. The anions, sulphate and chloride, are known to possess no emetic action. The possibility that the vomiting was due to contamination or ageing of the solution injected is precluded by the fact that in two of the three times the solution had been made up with freshly distilled water. The vomiting is the more unusual because of its complete absence during intravenous injection of magnesium in man, judging from a rather extensive observation of its use in treating the convulsions of uremia and eclampsia (9).

DISCUSSION. The sequence of various effects of magnesium are represented in figure 1. The changes of pulse rate appear almost at once, followed closely by the evidences of P-R and QRS changes. The next in sequence is respiratory failure. Heart block and cardiac arrest appear at the same or higher concentrations, i.e., only after the neuromuscular depression is well established. Indeed, they could not be demonstrated at all without artificial respiration. Thus in the therapeutic use of magnesium intravenously the avoidance of respiratory depression would seem to preclude the development of fatal cardiovascular toxic effects.

The action of magnesium on the heart differs markedly from that of potassium and from that of calcium. Potassium acts by completely disorganizing the ventricular complexes after a relatively slight elevation in concentration. The most marked disturbance is of intraventricular conduction although there may be some effect on the rest of the conducting system (8). Calcium induces a phase of increased automaticity often ending in ventricular fibrillation; if survived, this is followed by gradual arrest at higher levels, probably due to direct toxic action of calcium on the myocardium (4). Magnesium on the other hand primarily depresses all parts of the conduction system, sino-auricular, auriculo-ventricular and intraventricular. The most extreme effects on intraventricular conduction are, however, never so marked as to produce disorganization of the whole ventricular complex and death, as does potassium. The terminal cardiac arrest which always occurs if the magnesium concentration is pushed high enough cannot be explained as the result of this depression of conduction, since in most instances the last beats show a well defined and vigorous systole. Conduction of the impulse takes place in the usual way, though at a slower rate. For an adequate explanation of this final arrest it is necessary to assume some more direct toxic action of magnesium on the myocardium itself. Such an explanation is analogous to that which has been previously invoked to explain the final arrest of the heart when the concentration of calcium is increased to very high levels (4).

It would be desirable to correlate the depressant action of magnesium on the conduction system in the heart with its known effect on nervous tissue generally. Failure of simultaneous injection of calcium to prevent the depressant action of magnesium on the conduction time raises a question concerning the validity of such a correlation. Possibly the antagonistic action of calcium toward the effects of magnesium on the nervous system is exerted against certain phases only of that action. Further studies in this direction are desirable.

The sudden arrest of the heart in certain experiments at the actual moment of respiratory arrest, before artificial respiration could be initiated, suggests that asphyxia may increase the sensitivity of the heart to magnesium and so lower the concentration required to produce arrest. Attempts to test this hypothesis by interrupting the artificial respiration already established and observing the pulse rate for sudden bradycardia produced inconclusive results, so the suggestion must be considered unproven.

These observations are consistent with the older experiments of Hay (3) and Matthews and Jackson (5) and the more recent electrocardiographic observations of Rothberger and Zwillinger (7) and of Miller and Van Dellen (5a). They are also consistent with the observations of Neuwirth and Wallace (6). The suggestion by Zwillinger that intravenous injection of magnesium salts might be useful in cardiac therapeutics to suppress ventricular flutter and extrasystoles (10) receives scant physiological support from these experiments, since just such arrhythmias developed spontaneously or could be evoked in the presence of a greatly increased concentration of magnesium in the serum, and in fact after respiratory failure from magnesium. Theoretically it might be used to increase A-V conduction time, and to produce partial heart block in cases of auricular flutter and fibrillation. However, its transient action and its various associated toxic properties obviously render it much inferior to digitalis for all practical therapeutic use in such cases.

SUMMARY AND CONCLUSIONS

1. Continuous intravenous injection of magnesium sulphate in the unanesthetized dog produces a sequence of electrocardiographic changes affecting the heart rate and the intracardiac conduction.

2. The heart rate immediately increases as the concentration of magnesium in serum rises from 2 to 5 mEq. per liter. This initial tachycardia gradually gives way to a bradycardia.

3. Intracardiac conduction is depressed in all its elements, as seen from the following characteristic electrocardiographic changes: (i) Progressive increase in P-R interval beginning at a concentration of 5 to 10 mEq. per liter of magnesium in serum and continuing till death. (ii) Occasional

S-A and A-V block of various grades, occurring at levels greater than 15 mEq. per liter. (iii) Widening of QRS complex indicating an increase in intraventricular conduction time, beginning at 5 to 10 mEq. per liter.

4. Natural respiration disappears at 17 to 27 mEq. per liter. Occasionally cardiac arrest occurs simultaneously; more often under artificial respiration the heart continues to beat until a much higher concentration is attained.

5. Cardiac arrest may appear over a wide range, 17 to 66 mEq. per liter, but most frequently at 27 to 44 mEq. per liter. Normal systole is maintained till the end.

6. Cardiac arrest never precedes respiratory arrest.

7. Injection of magnesium in cats produces a sequence of changes similar to that observed in dogs.

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THE SENSITIZATION OF MOTONEURONES BY PARTIAL "DENERVATION"

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It has long been known that skeletal muscle, and also smooth muscle contracted by sympathetic impulses, become sensitized to chemical agents after degeneration of the nerve supply. Studies in this Laboratory have shown that there is a similar sensitization of smooth muscle relaxed by sympathetic impulses (Luco, 1937), of lachrymal glands (Maes, 1938), salivary glands (Pierce and Gregersen, 1937; Simeone and Maes, 1939), adrenal glands (Simeone, 1938), and the neurones of the superior cervical ganglion (conceived as innervated by preganglionic fibers), whether the denervation is partial or complete (Cannon and Rosenblueth, 1936; Simeone, Cannon and Rosenblueth, 1938; Rosenblueth and Cannon, 1939). This general law of denervation, especially as pertinent to the sensitizing of ganglion cells, suggested that the nerve cells of the brain and spinal cord might be sensitized if they were "denervated," i.e., deprived of nervous connections from which they routinely receive impulses. The research here reported was undertaken to learn about that possibility.

METHODS. Cats were used, weighing about 3 kgm. As a region for study the lower portion of the spinal cord was selected, for various reasons: 1, semisection interrupts ipsilateral descending fibers without noteworthy effects on the opposite side (Fulton, Liddell and Rioch, 1930); 2, the operation is relatively simple and is well borne; 3, a single muscle (quadriceps) attached to the patella and supplied by a readily accessible nerve can be easily isolated and used as an indicator of nervous discharges; 4, the direct blood supply to the muscle can be promptly and temporarily shut off while that to the cord is continued; and 5, injection into the aorta permits chemical agents to be delivered locally to both sides of the cord in a concentration not affecting the rest of the nervous system.

The serviceable semisections, *always* on the *right* side, were made, in different animals, between the 12th thoracic and 3rd lumbar vertebrae, most commonly between T12 and T13. In two instances a section as low as L4 damaged the control of the signaling muscle. After the spinal

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canal was well opened a fine needle, pressed directly downward, through the mid-line of the cord, served as a guide for a sharp blade of fine scissors which, on being closed, completed the semisection. Aseptic precautions and care in closing the wound prevented infection. Invariably the animals used the left hind leg and dragged the right.

According to Hoff (1932) about 4 days are required for degeneration of the "boutons terminaux" of the pyramidal fibers, at their points of contact on spinal neurones, after destruction of the motor cortex. To assure time for degeneration the interval between the semisection and the acute experiment ranged, in 27 of 35 cases, between 5 and 8 days; in 7 cases it was longer (up to 14 days). In 1 case it was only 2 days, which proved too short a period.

Since the experiment involved the possibility of sensitization of the partially denervated half-cord to chemical agents, avoidance of anesthetics was desirable, for they might be more effective on the sectioned than on the normal side. Accordingly, operations for the final tests were made as rapidly as possible under ether, whereupon the brain (including the medulla) was pithed through the foramen magnum and etherization was stopped; thereafter the animal was left for at least 30 minutes to permit artificial respiration to remove ether vapor.

For the acute experiment a strong thread was looped around the aorta just above the iliac branches and passed through a glass tube of small diameter; the mid-abdominal section was closed with a loose continuous suture; a simultaneous pull on the loop and push on the tube (which reached to the aorta) closed the direct blood flow to the leg muscles. Threads under the femorals allowed these nerves to be readily lifted and cut whenever desired. For injection into the aortic branches to the spinal cord the chest was opened on the left side between the 10th and 11th ribs, the ribs were separated 4 to 5 cm. by means of an adjustable spreader, the left lung was pushed upward and held away from the opening by dry absorbent cotton, the diaphragm was drawn away from the aorta, and when that vessel was cleared of its pleural covering a thread was looped around it, so that it could be lifted slightly for insertion of a syringe needle. Doubtless much of the injected fluid entered the splanchnic vessels; it seemed wise, however, to allow this escape rather than to prevent it by a deeply disturbing evisceration. In order to protect the thoracic structures a pad of cotton was laid over the opening in the chest wall, and removed only at times of injection.

For simplifying the muscular responses, most of the leg muscles were paralyzed by severance of the sciatic nerves. Drills were screwed into the two ends of the femur and so fixed in rigid clamps (supported on rigid uprights) that the bone was held vertical. The freed tendon of each quadriceps, attached by means of thick string to the short end of a firmly

supported lever, was stretched by a strong rubber band. The tension was approximately alike for the two muscles. The two levers had equal magnification—usually 10-fold. An electric heating pad kept the animal warm.

An important question was whether the intact side of the cord should be left intact or severed at the time of the immediate test. No influences could come from the brain, for that had been pithed. A number of reasons led to avoidance of any further operation, i.e., in completing semisection of the cord. 1. Equivalent effects were obtained on the two sides when the semisection was only two days old (a delay too brief for nerve degeneration) and the left side was not cut across. 2. In an experiment on an animal in which the right side of the cord had been severed at L2 for 8 days and the left for 1 day, the phenomena illustrated in figures 2 to 6 were repeated; that the centers on the less active (left) side were responsive was proved by lively "knee jerks" on that side. 3. The "knee jerk" (i.e., a *quadriceps* jerk evoked by a quick tap on the writing lever) was, as a rule, more easily evoked on the intact than on the cut side—a condition which indicated greater reflex responsiveness on the intact side. 4. In animals not previously operated upon, acute semisection was found to depress the irritability of the freshly separated side; such a depression, if produced by section of the still intact side at the time of testing, would favor the idea that the chronically semisectioned side is more sensitive than the other—the very idea which was suggested by previous evidence and which must not be favored. Confidence was felt, therefore, in the propriety of leaving intact the uncut side of the cord when the relative sensitiveness of the two sides was being examined.

As stimulating procedures, injections of strychnine sulphate, acetylcholine chloride (Merck) and sodium carbonate (half-saturated solution), and also asphyxia were employed.

The knee jerk was repeatedly used on each side during the testing process to make sure that the neuromuscular circuit involved had not been harmed by drugs or other experimental conditions. It is noteworthy that shortly after etherization was stopped the jerk was absent or slight on the cut side, while lively on the intact side; the increase of the response as minutes passed indicated that ether had had a more depressant effect on the partially isolated neurones than on the fully innervated contralateral neurones.

Of the 35 animals used 14 were not directly contributory: 5 of the first experiments, under dial, showed that a persistent anesthetic was unsatisfactory; in 3 there was incomplete severance of the right side of the cord; in 2 the left side was cut in the acute experiment before that was shown to be depressant; in 2 others the semisection was too low (at L4-5); in 1 there was damage to the left side when the right side was semisectioned; and in the remaining 1 an operation high in the thoracic cord gave equivocal results.

The other 21 experiments yielded results such as those reported in the following pages.

Incidentally, it was noted that a pull on the patellar tendon on the right side (e.g., when the string was being tied to the tendon), or a light pressure between thumb and finger on the right quadriceps, usually started a short clonic response—a phenomenon not commonly evident on the left (intact) side.

RESULTS. *Effects of strychnine.* Strychnine is an especially discriminative drug because there is evidence that it acts as an excitatory agent directly on the cell body of the neurone (Dusser de Barenne, 1912) and as a depressant at the neuromuscular junction (cf. Lanari and Luco, 1939). Any difference in the responses of the two quadriceps, therefore, is attributable to different effects on the severed and on the intact sides of the spinal cord.

Two types of responses were registered—single twitches and a more or less prolonged clonus. Strychnine (0.4 to 0.5 mgm.) injected into the aorta evoked one or other of these reactions. The contractions were more marked on the cut side. For example, after 0.4 mgm. there were 71 twitches in 15 minutes on that side and none, or the slightest signs of them, on the intact side.

More striking were the clonic contractions. These sometimes appeared on the two sides; in such instances they started later in the intact side, were intermittent, and were less persistent, ending, in one case, 47 minutes after the start, while the clonus on the cut side continued for 17 minutes longer. Injection of strychnine, in another animal, called forth, on the cut side only, a clonus which lasted 68 minutes; the contraction rate varied from 13 per sec. in the early stages to 14.5 in the later. At the rate of 13.5 per sec. this quadriceps contracted without intermission about 55,000 times (see fig. 1).

Strychnine was not much used by itself, at the start of an experiment, for overt stimulation, because its long-lasting effects interfered with testing other agents. It was occasionally employed, in small doses (0.1 mgm.) to render the responding neurones more sensitive to other stimuli.

In an animal with the lumbar cord fully isolated 0.2 mgm. of strychnine induced vigorous clonic contractions in the hind legs without affecting perceptibly the front of the body.

With strychnine, as with other convulsant drugs, there was sometimes an incidental general bodily movement which pulled slightly on both recording muscles and intruded on the typical record. Such a movement, however, raised both levers simultaneously; absence of coincident changes in the two records is proof that the action is local.

Effect of acetylcholine. Acetylcholine injected into the aorta might act in various ways. By direct stimulation it might cause the quadriceps to

shorten and thus might start a clonus. Or, just as it excites the ultimate autonomic neurones, it might excite spinal neurones; or it might depress them (cf. Schweitzer and Wright, 1937). These possibilities must be respected.

If at the testing time the femoral nerve on the side of the spinal semi-section (right) is severed, or if both nerves are severed, an intra-aortic injection of an adequate dose of acetylcholine evokes a contracture (since there are no action potentials) on the right side and little or no response on the left. In figure 2 are illustrative records, taken from an animal in which, shortly before the right femoral was cut, both knee jerks were quite active. This differential effect on the two muscles is greatly augmented by



Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 1. Termination of unilateral clonus lasting 68 minutes after an injection of strychnine. In this and other figures, upper record is from right quadriceps, controlled by partially denervated spinal neurones; lower, from left quadriceps, controlled by intact side of cord. All injections into lower thoracic aorta. Time, 1-minute and 1-second intervals.

Fig. 2. Acetylcholine (0.2 and 0.1 mgm.) injected after severance of right femoral nerve. Time in this and subsequent records, 5 seconds.

Fig. 3. Acetylcholine (0.2 mgm.) injected after aorta clamped above iliacs. At second signal, clamp off.

Fig. 4. Acetylcholine (0.3 mgm.) injected, nerves and blood supply intact. (Strychnine 0.1 mgm. previously injected.)

eserine. Evidently, when spinal neurones have been partially denervated, the muscle which they innervate becomes sensitized to acetylcholine.

The exaggerated response of the quadriceps on the semisectioned side raises the question whether such a response, when the femoral nerve is intact, may not be due to sensitization of the muscle to acetylcholine, without involvement of nerve cells in the cord. Two types of evidence indicate that acetylcholine stimulates specially the spinal neurones below the semisection—first, a contraction of the right quadriceps when direct blood flow to the legs is stopped, and second, a peculiar response different from that of the muscle alone.

In figure 3 is shown a quadriceps contraction following an injection of acetylcholine while the direct circulation to the hind leg muscles was

blocked by closure of the aorta just above the iliac branching, about 15 seconds before. Note the sharp response on the right side (upper record) and none on the left. The secondary effect is unexplained, though the depression between the two rises may be due to excess of the drug. That the left (intact) side was capable of responding was proved by presence of the knee jerk, and by a vigorous contraction when asphyxia was continued for 55 seconds. After the right femoral nerve had been cut, acetylcholine caused a slight contracture of the denervated quadriceps but had no effect on its companion.

The second kind of response was registered when acetylcholine was injected while the blood and nerve supplies to the quadriceps were not interrupted. It appeared to be a combination of the effects on the spinal neurones and on the muscle. As shown in figure 4, there is a quick contraction, similar to that recorded in figure 3, followed by a persistent contracted state in which clonic contractions rise from a curve like that of figure 2. It is possible that the clonus is started by a muscular contraction resulting from the direct effect of acetylcholine; the sharp initial rise in figure 4, as contrasted with the slow rise in figure 2, and also the presence of twitches on the intact side (lower record, fig. 4) indicate that in the circumstances the drug acts on the spinal neurones as well as on the muscle of the semisectioned side.

There may be some surprise that relatively large doses of acetylcholine (0.2 to 0.3 mgm.) were given in these experiments. It should be remembered, however, that no protective eserine was administered, and that doubtless much of the acetylcholine injected did not enter the small vessels leading to the spinal cord but escaped into the general circulation by way of the splanchnic arteries.

Incidentally, the observation may be reported that, if a clonus is being registered, an injection of acetylcholine (e.g., 0.2 to 0.5 mgm.) temporarily stops the rhythmic contractions as long as 15 to 20 seconds. If both quadriceps muscles are active, it may cause that on the side of the semi-sectioned cord to write a typical curve as in figure 2, without any superposed twitches, while sending that on the intact side into simple relaxation.

Effects of sodium carbonate. Strong solutions of sodium salts are known to have a convulsant effect by an action on the central nervous system (Münzer, 1898). An injection of a small amount (0.1 to 0.2 cc.) of a half-saturated solution of sodium carbonate into the thoracic aorta causes a discharge of nerve impulses. Figure 5 displays the result of 0.1 cc. injected while the aorta above the iliacs was closed. The marked muscular response on the right side (upper record) indicates that the stimulant action is especially pronounced on neurones partially denervated. The evidence that in this instance the sodium carbonate was affecting nervous elements and not the quadriceps (because the direct blood flow to the hind legs was blocked), was confirmed in other experiments in which larger doses of the

solution, after severance of the right femoral nerve, had no effect on the right quadriceps, but caused contraction of the left quadriceps, still connected to the spinal cord.

Repeated small doses (0.1 cc. or over) of the half-saturated solution of sodium carbonate appeared to have a depressant influence. After a few injections the stimulant action ceased; in one instance a large dose of strychnine (1.0 mgm.) thereafter was without issue.

Effects of asphyxia. The simplest way to excite spinal nerve cells is by partial asphyxia. It is so little disturbing that it can be repeated a number of times without much change in results. In our experiments asphyxia was easily induced by stopping artificial respiration. The effects were various. In some animals and in some tests, the discharge was solely on the cut side of the cord. In others it was on both sides, but then the discharge came earlier on the cut side, was more pronounced, and also more lasting, than on the intact side.

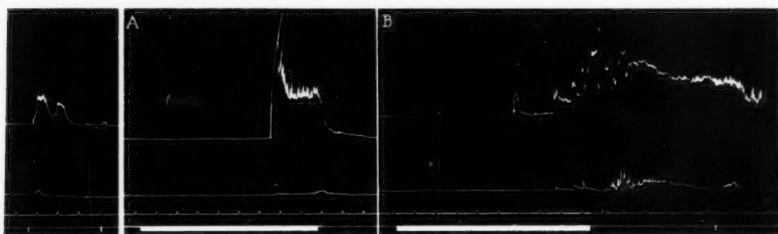


Fig. 5

Fig. 6

Fig. 5. Sodium carbonate (0.1 cc., half-saturated solution) injected, aorta clamped above iliaes. At second signal, clamp removed.

Fig. 6A. Asphyxia during period marked by broad line. B. Another type of response to same conditions, with aorta clamped. At second signal, clamp removed.

Figure 6A presents an example of pure unilateral effect. The quadriceps innervated by the partially isolated neurones was brought abruptly into contraction when the air was shut off for about 30 seconds, while the muscle controlled by the neurones on the intact side was hardly affected. This reaction was twice repeated within 8 minutes, once with the end of the aorta clamped. That the uncut side of the cord was fully capable of responding was proved by repeated tests of the knee jerk and also by a later clonus (accompanying a clonus of the cut side) which was induced by strychnine and acetylcholine.

Figure 6B shows the other type. Since in this case closure of the lower aorta asphyxiated the muscles without causing contractions, the excitatory effects which were recorded were clearly due to neuronal responses. Obviously the asphyxial state has had a much more prominent influence on the semisectioned than on the intact side of the cord.

DISCUSSION. The results reported in the foregoing pages and illustrated

in figures 1 and 3 to 6 reveal that in cats with brain pithed selected small doses of stimulatory drugs have a more pronounced effect on spinal neurones whose superior connections have degenerated (i.e., on partially "denervated" spinal neurones) than on corresponding neurones of the other (intact) side of the cord. From these results the inference may be drawn that as partial denervation of the nerve cells of the superior cervical ganglion cells (Simeone, Cannon and Rosenblueth, 1938) or the cells of the adrenal medulla (Simeone, 1938) sensitizes them to chemical agents, so partial denervation sensitizes spinal nerve cells to conditions which affect them. Thus, neurones in the central nervous system may be added to neurones of the autonomic system and also to glands, and smooth and skeletal muscle, as obeying a general law that degeneration of neurones which normally deliver impulses to a structure is attended by a lowered threshold to submaximal chemical stimuli applied to that structure.

In so far as the central nervous system is concerned the only references in literature pertinent to our observations, that we have found, have been in Claude Bernard's *Pathologie Expérimentale* (1880). He there states (p. 202) that Fouquier noted in cases of paraplegia that doses of strychnine too weak to cause convulsions in the normal limbs produced them in paralyzed limbs. Bernard also reports that strychnine administered to a frog with sectioned spinal cord produced convulsions in the posterior part of the animal before they appeared in the anterior part. Indeed, he goes so far as to express the opinion (p. 209) that "the excitability of all tissues seems to augment when they are separated from the nervous influence which dominates them." This suggestion is a remarkable example of the insight of a perspicacious physiologist, for at the time he made it he had very few facts to support it.

The effects of asphyxia in causing discharge of nerve impulses into the quadriceps (see fig. 6) are significantly related to observations by Russell (1893). In experiments on dogs and rabbits he found that in the early stages asphyxia (e.g., due to breathing nitrogen) caused exaggeration of the knee jerks, until a single tap on the patellar tendon evoked a clonic response. The increased excitability of the spinal cord under these conditions was not due to its release from cortical control, as might be surmised from depression or abolition of cortical excitability by asphyxia. Against that interpretation Russell had two arguments. He noted that when the knee jerks were exaggerated the excitability of the motor cortex was little, if at all, diminished, and that the exaggeration occurred quite as well when asphyxia was induced in animals whose spinal cords had been divided above the lumbar enlargement. His use of nitrogen to produce asphyxia showed that lack of oxygen, rather than increase of carbon dioxide, was the exciting condition.

A highly interesting and unexplained phenomenon is the sensitization

of skeletal muscle to acetylcholine by destruction of some penultimate (Hoff, 1932) neurones (fig. 2). Lanari (1936) has reported similar observations on men with lesions of the pyramidal tract, when acetylcholine was injected into the artery supplying the paralyzed muscles. This effect resembles the increase of responsiveness seen in smooth muscle when preganglionic fibers are severed. Hampel (1935) found the nictitating membrane was about half as much sensitized by cutting the penultimate (preganglionic) fibers as by cutting the ultimate fibers in the sympathetic supply.

Among observations requiring further attention is that illustrated in figure 3. Schweitzer and Wright (1937) reported that in unatropinized animals acetylcholine, even in smaller doses (i.e., 0.03 mgm. per kgm.) than we used, usually reduces the knee jerk. After atropine, small doses, they state, are rendered ineffective, but large doses have the usual inhibitory influence. When activity (e.g., a clonus) was going on we did note a depressant action of acetylcholine (see p. 736), but when the neuromuscular complex was inactive and the direct blood supply to the quadriceps was excluded, acetylcholine had an excitatory influence on sensitized spinal neurones (see fig. 3). Schweitzer and Wright used chloralose anesthesia, and we used no anesthesia; may chloralose alter the action of acetylcholine on spinal neurones?

SUMMARY

The sensitization of various structures to chemical stimulation, when their normal nervous connections have degenerated, raised the question whether the phenomenon was demonstrable in the central nervous system.

In cats the spinal cord was semisectioned above the sacral enlargement and time allowed for degeneration of descending fibers. In the acute experiment the brain was pithed, the quadriceps muscles were arranged for recording, and the thoracic aorta was prepared for injections.

Tests of relative sensitiveness of the neurones on the two sides of the cord, made by injecting strychnine (fig. 1), acetylcholine (figs. 3 and 4) and strong sodium carbonate solution (fig. 5), and by inducing asphyxia (fig. 6A and B), revealed greater effects on the semisectioned than on the intact side of the cord.

The inference is drawn that, as neurones of the superior cervical ganglion are sensitized by severance and degeneration of nerve fibers which routinely deliver impulses to them, neurones of the spinal cord are analogously sensitized by partial exclusion of their normal nerve connections.

Skeletal muscle is sensitized by section of penultimate motor neurones (fig. 2), thus resembling sensitization of smooth muscle sensitized by section of preganglionic sympathetic fibers.

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MEASUREMENT OF THE STROKE VOLUME OF THE HUMAN HEART FROM ROENTGENOGRAMS; SIMULTANEOUS ROENTGENKYMOGRAPHIC AND ACETYLENE-REBREATHING EXPERIMENTS

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The obvious importance of measuring the circulation rate or cardiac output in the intact man has stimulated many attempts to develop a satisfactory method. These are discussed in detail by Grollman (1932) but it is generally agreed that only the foreign gas principle has yielded acceptable and practical methods so far. Furthermore, it is generally agreed that of these latter methods, Grollman's (1929) acetylene method, especially in its later modifications, is both the most accurate and the most readily applicable procedure (Grollman, 1932; Grollman et al., 1933).

The acetylene rebreathing method is finding increasing applications (cf. e.g., Stewart et al., 1938), but the objections to it are not inconsiderable. The active and intelligent coöperation of the subject, who should have some preliminary training, is required. The experimental and analytical procedures—preparation and analysis of the gas mixtures, measurement of the metabolic rate and so on—are relatively tedious and difficult for routine use. It is still uncertain whether the circulation rate may not be altered in some subjects by the rather violent rebreathing that is necessary.

The cardiac output should be related to the difference between the volume of the heart in diastole and in systole. The volume of the heart has been estimated from x-ray films by many workers (cf. Roesler, 1937, chapter iv), but these measurements, made on teleroentgenograms or ortho-diagrams, refer only to diastole. With the multiple-slit roentgenkymograph (Stumpf, 1931, 1934; Stumpf et al., 1936) the positions of points at small intervals around the periphery of the heart are registered throughout one or more cardiac cycles. By this means it is possible to trace the outlines of the heart silhouette both in systole and in diastole (Keys and Friedell, 1938).

We have recently shown that the area of the silhouette tracing made from a frontal roentgenkymograph permits calculation of the heart volume

(Keys and Friedell, 1939). The average error in prediction of absolute heart volume by this means is less than 5 per cent; the greatest discrepancy was one case where the error was -15.9 per cent. Since the chief source of error is caused by abnormality of the relation between thickness of the heart (anterior-posterior) and the length and breadth of the heart, we may expect that the relative error in the comparison of systole with diastole will be very small. We have found this to be the case and the present paper presents the results of experiments on human subjects with whom we have made simultaneous roentgenkymograms and cardiac output measurements by the acetylene rebreathing method. We will show that the cardiac output can be calculated from the roentgenkymogram and that the disagreement with the acetylene method is scarcely greater than the error in the acetylene method.

Subjects. We used two groups of subjects classed according to clinical absence or presence of indications of valvular insufficiency. Most of the subjects in the series with normal valves were also normal in all other respects, but a number of experiments were made with cases of myxedema, glomerulonephritis with hypertension, and so forth. The subjects with valvular insufficiency included cases with varying degrees of aortic regurgitation, and mitral insufficiency, with and without hypertension. All subjects were given preliminary training in the form of dummy experiments on the day before experiment.

THE ROENTGENKYMOGRAPHIC METHOD. The roentgenkymograph consists of a grid of lead bars which is interposed between the subject and the x-ray film, and a mechanism which moves the film in its cassette at constant speed in the plane of the grid. In our kymograph the lead bars are 10 mm. wide and are separated by slits about 1 mm. wide. The total motion of the film during exposure is about 9 mm. We used exposures of 2.5 seconds so that 2 to 4 complete cardiac cycles were recorded. The distance from the target to the film was kept at 66 inches. With adults of average size good exposure is obtained with 100 milliamperes at 85 kilovolts potential using standard screens and cassettes.

We have used the frontal position throughout. The subject, seated on a revolving stool, faces the kymograph. At the signal he leans against the kymograph, takes a moderately deep breath and holds it for the total required 3 or 4 seconds.

A full discussion of the details of tracing the systolic and diastolic outlines will be given elsewhere but some explanation may be useful here. Figure 1 is a photograph of a typical multiple slit roentgenkymogram. The notches represent cardiac cycles; in general, the "peaks" (outermost excursions) are points on the periphery in diastole and the "valleys" are the corresponding points in systole. It is always easy to pick out a particular notch in which diastole and systole are clearly and definitely

indicated. The distance of the bottom of the valley from the base line is measured with dividers. (The base line is the bottom of the exposed strip in which the reference notch occurs.) Now this distance represents the same moment of time in each of the exposed strips and, accordingly, the moment of systole for the same cardiac cycle can be found for each strip. This distance is laid off on each strip on both sides of the heart. The points of intersection of the heart shadow with this distance (moment of time) represent points on the contour of the systolic projection of the heart. The diastolic outline is similarly traced, using as reference the outermost point or peak of the same notch taken for the systolic reference.

The completion of the outlines at the base and at the apex of the heart is made following the procedure of Bardeen (1918). The systolic and

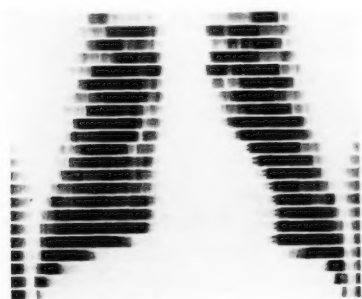


Fig. 1

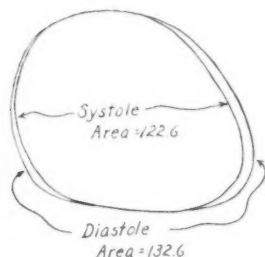


Fig. 2

Fig. 1. Photograph of a typical frontal roentgenkymogram of the chest. Subject of average adult size, time 2 seconds, distance 66 inches, 87 kilovolt peak potential, 100 milliamperes. This plate shows not quite 3 full contractions. Note difference in phase between the pulsation of the heart and the great vessels, particularly on the right side of the figure.

Fig. 2. Typical tracing of systolic and diastolic heart outlines in the frontal position made from a roentgenkymogram.

diastolic outlines are joined in these relatively uncertain regions. This does not introduce much error because there is relatively little contraction of the heart at either the base or the apex. This part of the tracing is facilitated by the fact that the upper and lower borders of the heart are more clearly visualized by the roentgenkymograph than by the ordinary x-ray film; phase differences in the movements readily distinguish the heart from the great vessels and the diaphragm.

The systolic and diastolic outlines as picked off on the roentgenkymogram are traced on tracing paper. A typical tracing of systolic and diastolic outlines in a normal heart is shown in figure 2. The areas of these tracings are then measured with a planimeter. Finally, these values are

corrected for distortion resulting from the divergence of the x-rays by the equation:

$$A = A_1 \frac{(y - c)^2}{y^2}$$

where A = true area, A_1 = observed area, y = distance from the x-ray tube target to the film, c = the distance from the outer margin of the heart (approximately the center in the lateral position).

We have routinely made lateral roentgenograms on all our subjects to measure precisely the distance of the heart from the kymograph film in order to correct for distortion. This precaution scarcely seems necessary, however, because the variations in the correction factor we find at this distance (66 inches film to target) are very small. The average correction for our experiments is about 12 per cent.

Calculation of volume. The equation for the calculation of the volume of the heart from the area of its corrected frontal silhouette under our conditions is $V = 0.63 (A)^{1.45}$ (Keys and Friedell, 1939). Other things being equal, the total stroke output of the heart would be:

$$I \quad \text{Stroke} = 0.63 (A_d^{1.45} - A_s^{1.45}),$$

where A_d and A_s are the diastolic and systolic projection areas respectively.

We recognized the probability that equation I would not hold without some correction owing, among other things, to our procedure of tracing diastole and systole as one line at the upper and lower borders of the heart, as well as the uncertainty about the anterior-posterior contraction. However, it seemed reasonable to expect that the true stroke volume would be simply related to the calculated stroke volume. In other words, we expected that we should be able to measure from the roentgenkymogram a volume difference which would be proportional to the true stroke volume. Accordingly, we made this calculation of equation I in all cases and used these results together with the values from the acetylene method to solve equation II:

$$II \quad \text{True stroke} = \text{function of kymograph stroke.}$$

ACETYLENE METHOD. We used the acetylene rebreathing method essentially as described by Grollman (1932). In most cases we took only 2 samples but in some experiments we used the 3 sample technique (Grollman et al. 1933); we found no difference in results when using 2 or 3 samples. The acetylene was purified by washing with cupric sulphate and bichromate acid solutions after which it was passed through a caustic soda tower as recommended by Grollman.

All analyses were made in duplicate, usually by two different analysts. The means of the duplicates were used for calculation but only rarely did the duplicates differ by as much as 0.05 per cent of the total gas volume.

Metabolism measurement. Metabolism was measured while the subject sat before the roentgenkymograph very shortly before the first roentgenkymogram was made. We used a Benedict-Roth machine and ran the tracing for 8 minutes.

Procedure for simultaneous kymography and acetylene rebreathing. In all but a very few cases the experiments were made in the early morning in a warm, quiet room and before breakfast. The subject, who had already been trained in the procedure, came to the experimental room and was stripped to the waist and seated on a revolving stool in front of the kymograph. After a period of more than 10 minutes' quiet rest the metabolism measurement was begun. As soon as the metabolism measurement was finished the subject was turned to face the kymograph and the first roentgenkymogram was made. He was then turned to the side again and the mouthpiece of the acetylene bag and the noseclamp were put in place. After breathing quietly through the side arm of the valve for about 2 minutes the acetylene rebreathing was carried out and immediately thereafter a second roentgenkymogram was made to provide a spare plate against accident and for comparison with the first film.

The entire procedure including two kymograms and the acetylene rebreathing was ordinarily completed in less than 5 minutes. Pulse rates were counted continuously during the acetylene rebreathing and before and after the kymograms.

Results from subjects with normal heart valves. The results from 25 measurements on 16 subjects with no evidence of valvular defects fit the equation:

Diastolic volume - systolic volume = $0.99 \times$ acetylene stroke output.
In other words equation II may now be combined with equation I:

$$\text{III} \quad \text{True stroke} = 1.01 \times 0.63 (A_d^{1.45} - A_s^{1.45}), \text{ or}$$

$$\text{IIIa} \quad \text{True stroke} = 0.64 (A_d^{1.45} - A_s^{1.45}).$$

The calculations are illustrated by table 1 which summarizes the results in four typical experiments selected at random. The correspondence between the stroke volumes calculated from the kymograms in this way with the stroke volumes obtained from the simultaneous acetylene-rebreathing procedure is shown in figure 3. The average difference between the two methods is ± 5.1 per cent and the greatest differences are $+ 10.7$ and $- 10.2$ per cent, referred to the acetylene method as standard. The coefficient of correlation between the two methods is $+ 0.984$.

In our studies on subjects with normal heart valves we have had uniformly good results, within the limits stated above, over a range of systolic volumes from 450 to 750 cc., and a range of strokes from 24 to 92 cc. It is notable that equally good results were obtained in cases with hypertension and with myxedema.

Results from subjects with aortic or mitral regurgitation. The acetylene method measures the net rate of blood flow through the lungs; we may call this the *effective circulation* and the amount per beat of the heart is the *effective stroke volume*. The roentgenkymographic method measures the amount of blood thrust out of the heart; we may call this the *gross stroke volume*. In subjects with effective valves these values are, as we have stated, equal. In subjects with aortic or mitral insufficiency, however, we might expect that the effective stroke volume will be less than the gross stroke volume and the difference would be a quantitative measure of the

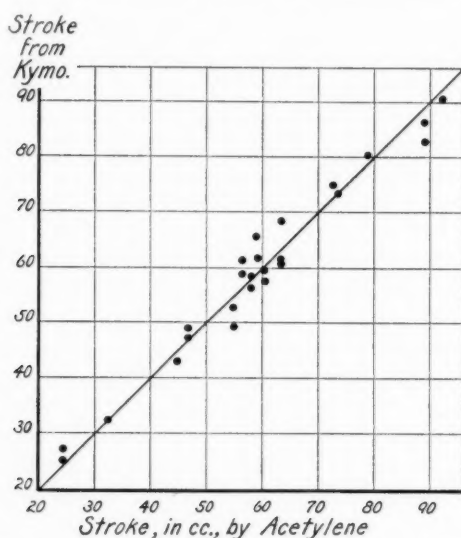


Fig. 3. Results of simultaneous roentgenkymography and acetylene rebreathing from subjects with normal heart valves. Kymogram strokes calculated from measurements of the frontal kymogram by the equation:

$$\text{Stroke} = 0.64 \left(\text{Area}_{\text{diast.}}^{1.45} - \text{Area}_{\text{syst.}}^{1.45} \right)$$

valvular efficiency. We have studied a series of such cases with simultaneous roentgenkymography and acetylene rebreathing and obtained results fully in accord with the theory.

The results from two typical cases are summarized in table 2. Subject "L. R. H." was diagnosed as "marked mitral insufficiency with minimal mitral stenosis and malignant hypertension." Subject "Dr. F. H." was diagnosed as "simple aortic insufficiency of moderate degree"; his resting blood pressure averages 140 systolic, 60 diastolic.

So far we have studied 13 subjects with aortic or mitral insufficiency;

with five of these cases we have made repeated studies on different occasions. In all these experiments the effective stroke volume, as measured

TABLE 1

Calculation of stroke volumes from roentgenkymograph tracings compared with results from simultaneous acetylene rebreathing experiments

Four typical experiments on normal subjects, selected at random. Abbreviations in the column headings are as follows: A_D and A_S = areas, in square centimeters, of the frontal projection of the heart in diastole and systole respectively; L = distance, in centimeters from the target of the x-ray tube to the film; C = total distance, in centimeters, from center of the heart to the film. For each subject the kymograph plates are given in order; the acetylene rebreathing was done between the exposure of these plates. Volumes are in cubic centimeters.

| SUBJECT | UNCORRECTED | | L | C | CORRECTED | | VOL. = $0.64 \times (A)^{1.45}$ | | STROKE VOLUME | |
|---------|-------------|-------|-------|------|-----------|-------|---------------------------------|-------|---------------|-------|
| | A_D | A_S | | | A_D | A_S | Diast. | Syst. | Kymo. | Acet. |
| D. Ho. | | | | | | | | | | |
| (1) | 114.9 | 104.9 | 167.6 | 8.8 | 103.2 | 94.3 | 532.0 | 466.9 | 66.1 | 59.2 |
| (2) | 114.0 | 104.5 | 167.6 | 8.8 | 102.4 | 93.9 | 526.1 | 469.1 | 61.9 | |
| A. Li. | | | | | | | | | | |
| (1) | 120.3 | 110.9 | 167.6 | 10.0 | 106.4 | 98.1 | 556.2 | 494.5 | 61.7 | 63.6 |
| (2) | 120.2 | 111.0 | 167.6 | 10.0 | 106.3 | 98.2 | 555.5 | 495.2 | 60.3 | |
| W. Ob. | | | | | | | | | | |
| (1) | 153.0 | 141.1 | 167.6 | 9.3 | 136.3 | 125.9 | 796.5 | 709.9 | 86.6 | 85.3 |
| (2) | 152.4 | 141.2 | 167.6 | 9.3 | 136.0 | 126.0 | 793.0 | 710.7 | 82.3 | |
| M. Ku. | | | | | | | | | | |
| (1) | 134.0 | 130.4 | 167.6 | 10.8 | 117.3 | 114.1 | 640.7 | 615.7 | 25.0 | 24.5 |
| (2) | 135.8 | 131.8 | 167.6 | 10.8 | 118.9 | 115.4 | 653.4 | 625.7 | 27.7 | |

TABLE 2

Calculation of stroke volumes and of valvular efficiency in a case of mitral insufficiency (L. R. H.) and in a case of aortic regurgitation (Dr. F. H.)

Abbreviations as in table 1

| SUBJECT | UNCORRECTED | | L | C | CORRECTED | | VOL. = $0.64 \times (A)^{1.45}$ | | STROKE VOL. | | LEAK AS % OF KYMO. |
|-----------|-------------|-------|-------|------|-----------|-------|---------------------------------|-------|-------------|-------|--------------------|
| | A_D | A_S | | | A_D | A_S | Diast. | Syst. | Kymo. | Acet. | |
| L. R. H. | | | | | | | | | | | cc. |
| (1) | 207.2 | 198.0 | 167.6 | 11.6 | 179.6 | 171.6 | 1188 | 1112 | 76 | 37.8 | 37.7 |
| (2) | 206.9 | 197.9 | 167.6 | 11.6 | 179.2 | 171.5 | 1184 | 1109 | 75 | | |
| Dr. F. H. | | | | | | | | | | | |
| (1) | 168.8 | 155.3 | 167.6 | 10.4 | 148.5 | 136.5 | 902 | 798 | 104 | 75.4 | 28.1 |
| (2) | 168.8 | 155.4 | 167.6 | 10.4 | 148.5 | 136.6 | 902 | 799 | 103 | | |

by the acetylene method, was definitely smaller than the gross stroke volume as measured by the roentgenkymograph. The calculated "leaks"

have ranged from 14 to more than 50 per cent of the gross cardiac stroke. Where the same subject has been studied on several occasions the results have been consistent. We do not propose to discuss these experiments and clinical studies here, but all our results so far are consonant with the belief that the combined x-ray and acetylene method can be used to measure, quantitatively, the amount of blood regurgitated through the aortic or the mitral valves.

Volume change and volume output of the heart. The validity of the roentgenkymographic measurement of cardiac output demands that the volume output of the heart per stroke must be some definite function of the change in area of the projection of the heart in the cardiac cycle. As we have shown, this is certainly the case, but the function found may seem somewhat unexpected. It is generally held that both ventricles contract simultaneously and, accordingly, we might expect that the stroke output, as calculated from the acetylene method, would be just half the volume difference of the heart from diastole to systole. Instead, the application of our area-volume equation indicates that the net output to the systemic circulation is normally very nearly exactly equal to the calculated difference in volume of the whole heart from diastole to systole.

A full quantitative explanation for the observed relation cannot be given as yet, but ample qualitative factors may be pointed out. In the first place, it must be remembered that the phases of the cardiac cycle are not absolutely synchronous in the ventricles and the auricles. During ventricular systole something like 20 per cent of the atrial filling takes place and the systolic volume of the whole heart will be greater by this amount than would be calculated from the ventricles alone. This means that, other things being equal, the difference between ventricular diastolic and systolic volumes should not be more than 1.6 times the acetylene stroke volume.

The roentgenkymograph does not register the total excursion of all the borders of the heart; only movements parallel to the slits and at right angles to the direction of motion of the film are recorded. We have used the kymograph in the vertical position so that the lateral excursions of the heart borders are registered. The lateral excursions probably reflect a larger share of the heart contractions than would be registered in any other direction, but the vertical component not registered must represent an appreciable amount of the contraction. This limitation of the kymograph, while it results in an underestimate of the volume alteration from systole to diastole, does not necessarily create any error. The lateral excursion should represent a definite component of the total excursion; this seems to be the case from the results recorded in this paper.

There are several other factors which might reduce the change in volume of the heart during a cardiac cycle. The simultaneity of contraction of the

two ventricles may not be as perfect as is usually believed. At the most, however, only a very small uncertainty could be allowed from this source—we estimate less than 10 per cent of the output of a single ventricle. Another source of discrepancy is the possibility that there is, during contraction, a change in the anterior-posterior shape of the heart relative to the frontal shape. Technical limitations prevent us from checking this point by means of roentgenkymograms taken in the lateral position but even if there is such a change it must be small and it would be difficult to measure.

Basal cardiac output. It is frequently assumed that "basal" cardiac output is attained as readily as the basal metabolic state and that, in addition to the requirements of rest and absence of digestion, all that is

TABLE 3

Reduction in cardiac output in the resting state in successive experiments made at intervals of about a week

In all cases these normal subjects were given full training trials on the day before the first experiment. Mean stroke outputs in cubic centimeters.

| SUBJECT | SUCCESSIVE EXPERIMENTS KYMOGRAPH ONLY | | | | |
|--------------|--|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 |
| D. V. R..... | 83.0 | 80.0 | 82.6 | 67.3 | 64.9 |
| F. P. S..... | 55.0 | 47.6 | 39.7 | | |
| W. A..... | 121.1 | 91.0 | 84.8 | 85.5 | 85.5 |
| | KYMOGRAPH AND ACETYLENE | | | | |
| | | | | | |
| J. M..... | 92.4 | 72.6 | 60.4 | | |
| A. L..... | 63.6 | 55.0 | 47.2 | | |
| W. O..... | 85.3 | 63.7 | | | |
| M. K..... | 32.0 | 24.5 | | | |
| D. H..... | 59.2 | 42.3 | | | |

necessary is a "training trial" on the day before. We found this to be decidedly not the case with our kymographic technique as well as with the acetylene method and with our simultaneous method. We carried out repetitions of the measurements by the several methods at intervals of a week or so on a number of subjects selected for general lack of excitability. In spite of the fact that in all cases these subjects were given a full training trial on the day before the first experiment, the stroke volume almost always became much smaller with each succeeding experiment, up to the third or fourth experiment. These results are given in table 3. They show clearly that, under our experimental conditions at least, it is impossible to say anything about basal cardiac output unless the subject has had a considerable period of "training."

Error of the roentgenkymographic method. We have already shown (Keys and Friedell, 1939) that the mean error in prediction of the heart volume from the frontal projection area measured on the x-ray plate is only about 5 per cent and rarely is as great as 10 per cent. Eighty per cent or more of this error is referable to abnormality in the shape of the heart. Since any shape abnormality must be presumed to persist both in systole and in diastole, the error in the estimate of stroke volume from the kymogram must be approximately the same, in percentage terms, as the error in the estimate of total heart volume.

In this paper we show that the disagreement between the kymographic estimate and the calculation from the acetylene method is only of the same order of magnitude as the error in estimation of heart volume from projection area in cadavers. This might imply that the acetylene method is perfect, which certainly is not true; in our experience successive measure-

TABLE 4
Apparent "efficiency" of the valves of the heart of normal subjects as judged by repeated measurements

| SUBJECT | EFFICIENCY, ACET. AS % OF KYMO. | | | | | MEAN |
|------------|---------------------------------|-----|-----|-----|-----|------|
| | 1 | 2 | 3 | 4 | 5 | |
| J. M. | 111 | 103 | 98 | 106 | 102 | 104 |
| A. L. | 103 | 107 | 114 | 106 | | 107 |
| W. O. | 100 | 104 | 93 | | | 99 |
| M. K. | 99 | 100 | 90 | | | 96 |

ments of cardiac output by the acetylene method may differ nearly as much as a kymograph result differs from the simultaneous acetylene method determination. In other words, our results with the simultaneous procedure are generally rather better than expected. It seems probable that one reason for this is that shape abnormalities in the living heart are less frequent than in the hearts of cadavers. This might mean that the roentgenkymographic method is more precise than the acetylene method.

Apart from these smaller errors the occurrence of grossly incorrect results must be considered. In several hundred experiments with the kymographic method no case of a palpably gross error has been encountered. This may be contrasted with the acetylene method with which our experience is that gross errors occur in from 5 to 10 per cent of experiments. We consider that a gross error has been made where, in a resting experiment, the cardiac index is less than 70 per cent or more than 150 per cent of the normal average.

The results in figure 3 represent single determinations and in several cases there is a discrepancy of 10 per cent between the acetylene stroke

volume and the kymographic measurement. We have made a few repeated studies on a number of normal subjects to see whether this sort of discrepancy represents error or whether there are constant individual differences of this magnitude. The results are given in table 4. Apparently errors account for a major part of these larger discrepancies, but there is also an indication that personal peculiarities may produce results that are consistently slightly aberrant.

DISCUSSION. The excursion of the left border of the heart as registered on the roentgenkymogram was used by Johnson (1937) in an attempt to obtain a relation to the stroke as estimated from a dye injection method. Apart from the well-known inaccuracy of dye injection methods, the kymographic calculation used by Johnson is open to very serious objections. Measurements confined to one side of the heart include pendulum motion and the attempt to estimate the volume of a single ventricle involves a number of assumptions which cannot be more than qualitatively acceptable. The values for the stroke volume given by Johnson (*op. cit.*, p. 170) are very far from the usual normal levels; in any case discrepancies up to 40 per cent in 8 measurements on 4 normal subjects do not inspire confidence. Johnson's studies are valuable and interesting, however, in that they show a rough relation between the x-ray excursion and an estimate of stroke volume.

SUMMARY

A method is described in which the volume stroke of the human heart is estimated from measurements of the areas of the systolic and diastolic outlines of the heart in the frontal position on a roentgenkymographic film.

Roentgenkymograms were made simultaneously with acetylene re-breathing experiments. The results from 25 experiments on 16 normal subjects fit the equation:

$$\text{Stroke volume} = 0.64 (\text{Area}_{\text{diastole}}^{1.45} - \text{Area}_{\text{systole}}^{1.45}).$$

The average difference between the two methods was ± 5.1 per cent and the greatest differences were $+ 10.7$ and $- 10.2$ per cent, referred to the acetylene method as standard. Similar results were obtained with patients with circulatory abnormalities (myxedema, nephritis, hypertension) so long as no valvular defects were present.

Patients with valvular leaks (mitral insufficiency, aortic regurgitation) always have stroke volumes, measured by the kymograph, which are larger than the true stroke volume and this discrepancy is parallel to the best judgment of the leak from clinical studies. We believe that comparison of results from the two methods gives a quantitative measure of the valvular defect.

It is noted that basal cardiac output as measured by either roentgenkymography or acetylene rebreathing generally is not attained until the subject has been through the procedure at least 3 or 4 times on different occasions.

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INFLUENCE OF ADRENALECTOMY ON THE BLOOD AND URINE KETONES DURING FASTING AND ANTERIOR PITUITARY EXTRACT ADMINISTRATION

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Adrenalectomy has been reported to produce a marked reduction in the ketonuria following pancreatectomy (1), phloridzin poisoning (2), pregnancy (3), fasting (5) and the administration of anterior pituitary extracts (4, 5, 6). In every case the reduction in ketonuria was attributed to reduced ketone body formation in the organism. Mirsky (7) has now presented evidence that the effect of adrenalectomy upon the ketonuria, in so far as anterior pituitary ketogenesis in the rat is concerned, is due almost entirely to the elevation of the renal threshold for ketone bodies. This has led us to reexamine our earlier experiments in this light. When they were carried out a good ketone method for small quantities of blood was not available although a method which was being developed did show (5) that anterior pituitary extract could raise the blood ketones in adrenalectomized animals. We have made the blood ketone determinations in these experiments by the procedure of Barnes (8) which in its present form is specific, accurate and rapid. To obtain blood for the ketone determinations the animals were anesthetized with pentobarbital sodium and an oxalated specimen was drawn from the abdominal aorta as the animal was sacrificed. Other methods were those which we have used in ketone body studies before (5). Essential details of the experiments are incorporated in the tables.

Experiments 2, 3, 4 (table 1), 6, 7, and 8 (table 2) confirm Mirsky's (7) conclusion that adrenalectomy raises the renal threshold for the excretion of ketone bodies. This heightening of the renal threshold is undoubtedly the explanation of some of the results published by ourselves and others in which only the ketonuria was used as a measure of ketosis. Contrary to our earlier conclusion (5) there is now no evidence that adrenalectomy may profoundly affect the ketosis of fasting (expts. 1, 2, 3 and 4—table 1; expt. 6—table 2). Although the blood ketones may at times be considerably lower in fasting adrenalectomized rats than in comparable controls (expts. 2 and 4—table 1) there is some question as to the interpre-

tation which may be placed upon differences in blood levels, a point which will be considered shortly in more detail. Although we have not found that the blood ketone level is always lower after adrenalectomy none of our

TABLE 1

| NUMBER RATS | AVERAGE BODY WEIGHT | FASTING PERIOD | TIME AFTER ADRENAL-ECTOMY | BLOOD KETONES | URINE KETONES--MGM. PER RAT PER DAY | | | | |
|---|---------------------|----------------|---------------------------|---------------|-------------------------------------|------------|-------------|-------------|-------------|
| | | | | | 0-12 hours | 0-24 hours | 24-36 hours | 24-48 hours | 48-72 hours |
| Experiment 1. Males on a diet* low in protein for 11 days prior to fasting | | | | | | | | | |
| | gm. | hours | hours | mgm. per cent | | | | | |
| 4 | 198 | 48 | Controls | 30.1 | | | | | |
| 4 | 198 | 48 | 24 | 25.2 | | | | | |
| 4 | 214 | 72 | Controls | 30.7 | | | | | |
| 4 | 214 | 72 | 48 | 26.7 | | | | | |
| 4 | 212 | 96 | Controls | 36.9 | | | | | |
| 4 | 209 | 96 | 72 | 30.6 | | | | | |
| Experiment 2. Males receiving only 50 per cent sucrose solution for 6 days prior to fasting | | | | | | | | | |
| 3 | 263 | 72 | Control | 23.0 | | 3.1 | | 48.0 | |
| 3 | 262 | 72 | 48 | 15.7 | | 0.1 | | 11.0 | |
| 3 | 257 | 96 | 72 | 33.9 | | 0.5 | | 4.2 | 6.1 |
| 3 | 268 | 96 | Control | 38.4 | | 4.0 | | 12.0 | 93.0 |
| Experiment 3. Males on a diet* low in protein for 21 days before the experiment | | | | | | | | | |
| 9 | 202 | 120 | Controls | 40.7 | | 1.0 | | 15.0 | 20.2 |
| 9 | 199 | 120 | 96 | 42.3 | | 0.8 | | 7.6 | 15.8 |
| Experiment 4. Females fasted from the stock diet | | | | | | | | | |
| 3 | 158 | 36 | Controls | 13.0 | 10.0 | | | | |
| 3 | 165 | 36 | 12 | 15.2 | 2.0 | | | | |
| 3 | 172 | 48 | Controls | 14.5 | | 3.1 | | | |
| 3 | 169 | 48 | 24 | 15.0 | | 0.8 | | | |
| 3 | 175 | 60 | Controls | 34.5 | | 3.0 | 28.4 | | |
| 3 | 184 | 60 | 36 | 10.1 | | 1.2 | 4.0 | | |
| 3 | 196 | 72 | Controls | 40.2 | | 2.0 | | 28.2 | |
| 3 | 178 | 72 | 48 | 25.4 | | 0.8 | | 1.5 | |

* The low protein diet was composed of casein 5, sucrose 48, Standard salt mixture (Osborne and Mendel) 5, Yeast Vitamine powder (Harris Laboratories) 2, cod-liver-oil 5 and Crisco 35.

results support the contention of Thaddea and Kühn (9) that adrenalectomy leads to an increase in the level of ketone bodies in the blood.

Mirsky (7) reported a slightly higher blood ketone level in adrenalectomized rats treated with an anterior pituitary extract prepared according

TABLE 2

| NUMBER RATS | SEX | AVERAGE BODY WEIGHT | FASTING PERIOD | TIME AFTER ADRENAL- ECTOMY | TIME AFTER ADMIN. ANT. PIT. EXT. | BLOOD KETONES | URINE KETONES—MGM. PER RAT PER DAY | |
|----------------|-----|---------------------------|-------------------|----------------------------------|--|------------------|---------------------------------------|----------------|
| | | | | | | | 0-24 hours | 24-48 hours |

Experiment 5. On the low protein* diet prior to fasting. 3 cc. of the anterior pituitary extract was injected subcutaneously into each rat

| | | gm. | hours | hours | hours | mgm. per cent | | |
|---|---|-----|-------|----------|-------|------------------|--|--|
| 2 | ♀ | 175 | 48 | Controls | 5 | 58.0 | | |
| 2 | ♀ | 169 | 48 | 48 | 5 | 72.1 | | |
| 2 | ♀ | 170 | 48 | Controls | 9 | 59.4 | | |
| 2 | ♀ | 173 | 48 | 48 | 9 | 72.2 | | |

Experiment 6. Fasted from the stock diet. Each rat was given 1 cc. of the anterior pituitary extract at the end of 48 and 60 hours of fasting

| | | | | | | | | |
|---|---|-----|----|----------|----|------|--|------|
| 2 | ♀ | 168 | 36 | Controls | | 12.5 | | |
| 2 | ♀ | 172 | 36 | 12 | | 15.2 | | |
| 2 | ♀ | 184 | 48 | Controls | | 14.5 | | |
| 2 | ♀ | 188 | 48 | 24 | | 14.8 | | |
| 2 | ♀ | 191 | 60 | Controls | 12 | 34.7 | | 12.1 |
| 2 | ♀ | 178 | 60 | 36 | 12 | 10.3 | | 0.6 |
| 2 | ♀ | 169 | 72 | Controls | 12 | 39.8 | | 22.7 |
| 2 | ♀ | 173 | 72 | 48 | 12 | 25.7 | | 1.6 |

Experiment 7. Fasted from the low protein* diet. Dose of anterior pituitary extract 3 cc. per rat

| | | | | | | | | |
|---|---|-----|----|---------|---------|------|--------|--|
| 3 | ♂ | 173 | 48 | Control | Control | 43.5 | } 44.0 | |
| 3 | ♂ | 179 | 48 | Control | 5 | 49.6 | | |
| 3 | ♂ | 169 | 48 | 48 | Control | 35.4 | } 28.0 | |
| 3 | ♂ | 174 | 48 | 48 | 5 | 46.6 | | |
| 3 | ♀ | 158 | 48 | Control | Control | 40.0 | } 50.2 | |
| 3 | ♀ | 154 | 48 | Control | 5 | 55.2 | | |
| 3 | ♀ | 163 | 48 | 48 | Control | 44.3 | } 40.3 | |
| 3 | ♀ | 158 | 48 | 48 | 5 | 56.4 | | |

Experiment 8. Fasted from a complete** diet. Dose of extract as in experiment 7

| | | | | | | | | |
|---|---|-----|----|---------|---------|------|--------|--|
| 3 | ♂ | 231 | 48 | Control | Control | 32.8 | } 15.1 | |
| 3 | ♂ | 239 | 48 | Control | 5 | 37.5 | | |
| 3 | ♂ | 226 | 48 | 48 | Control | 19.3 | } 6.2 | |
| 3 | ♂ | 232 | 48 | 48 | 5 | 22.6 | | |
| 3 | ♀ | 181 | 48 | Control | Control | 33.5 | } 12.7 | |
| 3 | ♀ | 190 | 48 | Control | 5 | 38.4 | | |
| 3 | ♀ | 183 | 48 | 48 | Control | 21.4 | } 6.1 | |
| 3 | ♀ | 188 | 48 | 48 | 5 | 35.0 | | |

** The regular diet was composed of casein 20, sucrose 35, dried powdered brewer's yeast 10, Standard salt mixture 5, cod-liver-oil 5 and Crisco 25.

to Best and Campbell (10) than in similarly treated controls. We have obtained similar results (expt. 5—table 2) but no difference or a lower level in the adrenalectomized rats was more common (expts. 6, 7, and 8—table 2). The time after giving the extract is probably an important factor in the result. The curve due to an anterior pituitary extract ketogenesis may be very different in adrenalectomized and normal animals. In any case the conclusions (4, 5, 6) that adrenalectomy greatly reduces or abolishes the ketosis due to anterior pituitary extracts were incorrect.

We may question whether or not adrenalectomy has ever been shown to influence ketogenesis. It has been found that the ketonuria may not indicate the true situation concerning the extent of a ketosis. For that matter the ketonemia does not necessarily show its magnitude either for the blood ketone level is really the difference between the production on the one hand and utilization and excretion on the other. If conditions could be obtained in which ketone body utilization was more or less constant in the broad sense fluctuations in the blood ketone concentration would reflect variations in the rate of ketogenesis.

In an earlier publication in the *Journal* (11) the conclusion was reached from observations on the excretion of ketone bodies in the urine of rats fed the sodium salt of B-hydroxybutyric acid that adrenalectomy increased the ketolytic activity of the organism. The fact that adrenalectomy alters the renal threshold for ketone bodies renders this conclusion invalid. Even though adrenalectomy might leave the renal threshold for the ketone bodies unaltered our conclusion is still invalid for the ketonuria in those experiments was not dependent simply upon the amount of the fed ketone body which was not oxidized by the organism as we then thought. The ketonuria of fasting rats fed sodium B-hydroxybutyrate is determined by the sum of the fasting ketosis plus the ketone bodies fed plus the ketogenic action of the alkali left when some of the fed acid is oxidized. Obviously it cannot be used to measure ketolytic activity. The influence of adrenalectomy upon ketolytic activity will have to be studied by use of arterio-venous differences (12) and the rate of utilization of administered ketone bodies by the liverless animal which cannot produce these substances.

SUMMARY

Adrenalectomy leads to a rise in the renal threshold for the ketone bodies so that the urine may be almost or entirely free of these compounds while the blood ketone levels are very high.

Assuming a reduction in the blood ketone level to be due to less ketogenesis and not increased ketone body oxidation the blood ketone levels after adrenalectomy indicate only a slight lowering of ketone production during fasting after the removal of the glands.

Studies of the blood ketone levels as well as the urine excretion show little effect of adrenalectomy upon the ketogenic action of anterior pituitary extracts.

The difficulties involved in the use of the urine excretion rates or blood ketone levels as measures of ketolysis or ketogenic activity are pointed out.

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THE RELATIONSHIP BETWEEN THE CENTRAL NERVOUS SYSTEM AND THE REPRODUCTIVE CYCLE IN THE FEMALE GUINEA PIG¹

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It has been shown recently that the estrin-progesterone induced sexual behavior in the ovariectomized guinea pig can occur after removal of all brain tissue rostral to the anterior margins of the superior colliculi and the mammillary bodies, and that this behavior can be abolished by a transection of the brain-stem extending from the anterior margin of the superior colliculi to the caudal margin of the mammillary bodies. Furthermore, it has been found that sexual behavior may be abolished permanently by destruction of an afferent pathway which runs through the posterior quadrants of the spinal cord, crosses in the medulla and runs through the tectum (Dempsey and Rioch, 1939).

These experiments, while demonstrating that the behavioral changes induced by the ovarian hormones are mediated by the central nervous system, provide no information as to whether the reproductive cycle and ovulation in the normal animal are also influenced by the nervous system. The assumption that the reproductive cycle is influenced by nervous control is supported by the following facts. 1. Changes in the period of illumination cause changes in the time at which heat occurs (Dempsey, Myers, Young and Jennison, 1934), rats (Browman, 1936; Hemmingsen and Krarup, 1937), and ferrets (Bissonnette, 1933). 2. Cyclical changes in the ovary of the guinea pig still occur after prolonged injection of large quantities of estrin (Dempsey, 1937). 3. Section of the pituitary stalk in the rabbit abolishes ovulation, which normally occurs after copulation (Brooks, 1937), and lesions in the nervous system of the ferret abolish the stimulation of the reproductive cycle incident to increased illumination (Clark, McKeown and Zuckerman, 1939). 4. Direct electrical stimulation of the brain of estrous rabbits causes ovulation (Marshall and Verney, 1936). 5. Lastly, it has been stated, on the basis of transplantation experiments, that interruption of the nervous supply to the pituitary results in failure of the release of luteinizing hormone, and therefore of ovulation, in the guinea pig (Schweitzer, Charipper and Haterius, 1937).

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It seemed advisable, in view of the observations cited above, to investigate the effect of lesions at various levels of the nervous system on the reproductive cycle and ovulation in a spontaneously ovulating mammal. The present paper is a report of such experiments on the female guinea pig. In addition, certain observations are included which supplement a previous report on the nervous control of sexual behavior (Dempsey and Rioch, 1939).

MATERIAL AND METHODS. Brain lesions were produced surgically under anesthesia in adult female guinea pigs. The tissue to be removed was either cut out with a sharp spatula or sucked out through a fine glass pipette attached to a suction pump. In one series of observations the reproductive cycle was followed in intact animals by observation of the opening of the vaginal closure membrane, and ovulation was determined by laparotomy and direct inspection of the ovaries. In another series of experiments brain lesions were produced in animals from which the ovaries had been removed previously. In these animals sexual behavior was observed after injections of estrin followed by progesterone,² a procedure which in normal animals is invariably followed by heat (Dempsey, Hertz and Young, 1936).

Hypophysectomy, section of the pituitary stalk and lesions in the posterior hypothalamus were performed by means of the parapharyngeal approach to the pituitary of the guinea pig, devised by McPhail and Parkes (1933).

Upon killing each animal, the brain was removed and fixed in 10 per cent formalin. The extent of the lesion was determined by gross dissection or by inspection of 35 μ serial sections stained in thionin. In the case of the pituitary stalk operations the skull and brain were fixed *in situ* and serial sections through the sella were prepared. In hypophysectomized animals the skull and brain were also fixed *in situ* in 10 per cent formalin. Subsequently, the brain tissue was removed by careful dissection under a binocular dissecting microscope. In this way the sella turcica was exposed and examined carefully for remaining fragments of the pituitary gland. If there was any doubt the sella was decalcified and serially sectioned for subsequent histological examination.

RESULTS. *Cortical and striatal lesions.* It has been impossible, so far, to prepare chronic, completely decorticate guinea pigs, due to the anorexia which develops, coupled with our inability to feed personally the large quantities of food normally consumed by this species. Ovulation was observed, however, in two hemidecorticate animals and in one three-quarter decorticate in which only one motor and frontal area remained. One animal in which the motor and frontal areas had been bilaterally

² The hormone preparations used in this work were supplied through the courtesy of Dr. Erwin Schwenk of the Schering Corporation.

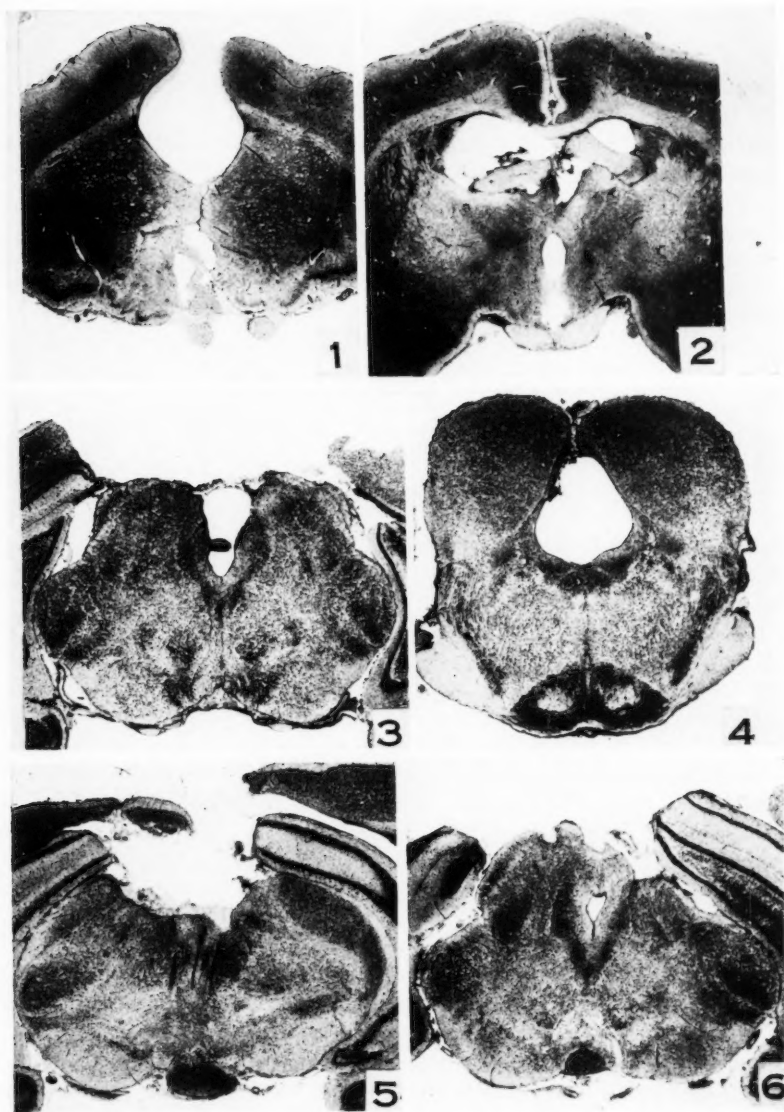
removed did not ovulate during the 4 months which it survived. Furthermore, two animals were prepared by injections of 0.05 I.U. progesterone (Proluton, Schering) daily for 15 days. The injections were then discontinued and the cortex was bilaterally removed from these animals 2 days later. Ovulation did not occur during the 4 and 5 days, respectively, which these animals survived the operation, although in normal animals discontinuance of such progesterone injections is followed by ovulation after 2 or 3 days (Dempsey, 1937).

Lesions were produced by means of the sucker in the septal area of two animals. In one animal the vaginal closure membrane did not open and ovulation as determined by repeated laparotomies did not occur during a period of 2 months following the operation. In the other animal, in which the lesion was more extensive than the first, ovulation occurred at 16- to 17-day intervals for 4 months. Figures 1 and 2 show that in this instance the lesion had completely destroyed the septal area, had interrupted the fornix and anterior commissure, and had destroyed the rostral portion of the preoptic area and the medial half of the tuberculum olfactorium.

Mesencephalic and diencephalic lesions. In three animals the mesencephalon was partially transected at the level of the inferior colliculi to the depth of the aqueduct of Sylvius. This lesion, while it abolishes sexual behavior and the evading reactions caused by rubbing or stroking the hair on the back and rump of the animal (Dempsey and Rioch, 1939), does not interfere with the ovulatory mechanism in any way, since all these animals ovulated repeatedly at 15- to 18-day intervals during the 2 to 4 months which they survived the operation. Likewise, ovulation occurred in an animal in which the tectum was divided in the midline, extending completely between both inferior and superior colliculi to the depth of the aqueduct (figs. 3 and 4). The ovaries were then removed and injections of estrin and progesterone were made. Completely normal sexual behavior was observed, and during the intervals between injections the evading responses were also normal.

The area pretectalis was removed in two animals by means of the sucker. Following the operation ovulation occurred in both animals. The ovaries were then removed and the animals injected with estrin and progesterone. In both, typical estrous behavior occurred and, during the intervals between injections, evading responses could be obtained. Examination of the brains of these individuals revealed that the lesion had successfully removed the pretectal area. In one, the habenular nucleus of the thalamus was also damaged, while in the other the lesion extended somewhat more caudad and had destroyed the anterior margin of the superior colliculi as well as the pretectal area (figs. 5 and 6).

Lesions were also produced in the mammillary bodies. The pituitary was exposed by the parapharyngeal approach, its caudal pole elevated and



Figs. 1 and 2. Transverse sections through the region of the optic chiasm of the brain of a guinea pig in which the septal regions had been removed and the preoptic area injured. Mating behavior, evading responses and ovulation were observed after the operation. Thionin, $\times 8$.

Figs. 3 and 4. Sections through the brain of a guinea pig in which the tectum had been divided in the midline to the depth of the aqueduct of Sylvius. Mating behavior, evading responses and ovulation were normal after the operation. Thionin, $\times 6$.

Figs. 5 and 6. Sections through the brain of a guinea pig in which the pretectal area and the anterior margin of the superior colliculi had been removed. Mating behavior, evading responses and ovulation were normal after the operation. Thionin, $\times 6$.

retracted, the dura incised and the brain tissue sucked out by means of a small curved pipette. Following the operation both animals showed evading reactions. One animal died a few days after the operation, but ovulation was observed in the other before it was killed a month later. Examination of the brains showed that the mammillary bodies had been removed completely in the animal which died first (figs. 7 and 8), while in the other the anterior one-quarter of these structures remained intact.

Section of the pituitary stalk. In a series of nine animals the pituitary was exposed by the parapharyngeal approach and the rostral pole was elevated and retracted, thus exposing the posterior lobe and the pituitary stalk. The pituitary stalk was then divided by the use of either a very fine suction pipette or a small cutting hook which was passed completely around the stalk and then retracted. All steps of the operation were carried out under conditions of perfect visibility through a modified Zeiss binocular at a magnification of 3X. The bleeding which occurred when the stalk was severed was usually negligible and in all cases was easily controlled by slight pressure with a small cotton sponge.

After the operation four of the nine animals were observed to ovulate during the 1 to 6 months they were allowed to survive (figs. 9 and 10). In two of the four the vaginal closure membrane did not reopen and ovulation did not occur for 2 and 3 months, respectively, after the operation. Following this period of suspended reproductive activity, however, ovulation occurred cyclically until termination of the experiment. In the other two ovulation was never interrupted by section of the pituitary stalk. In one of these two the superior cervical sympathetic ganglia were subsequently removed bilaterally. Thereafter, ovulation likewise occurred at 17-day intervals for two periods, at the end of which the animal was sacrificed.

In the five remaining animals of this series the vaginal closure membrane never reopened and ovulation did not occur before termination of the experiment 1 to 6 months after the operation.

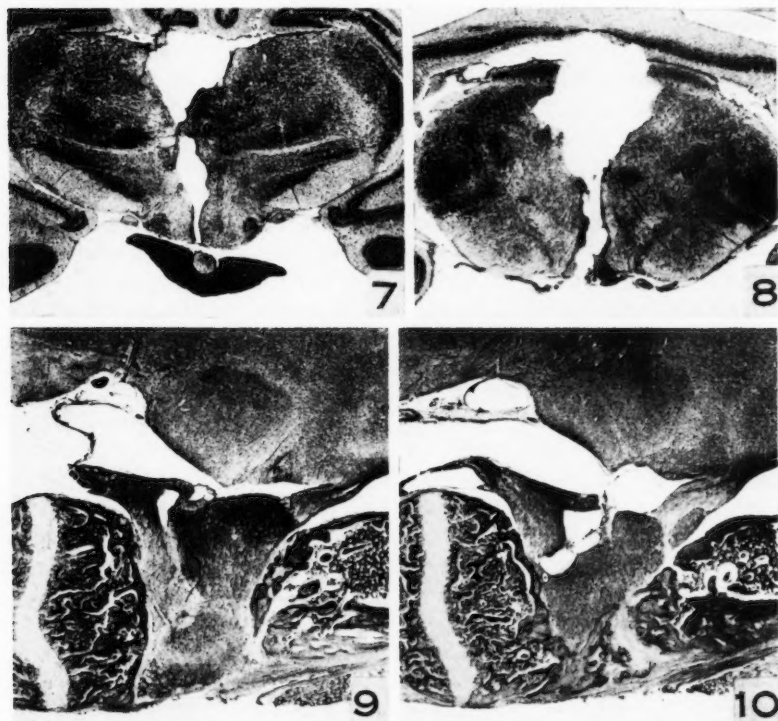
Four of these nine animals, one of which had not ovulated and three of which had ovulated, were subsequently ovariectomized and injected with estrin and progesterone. Estrous behavior was observed after the injections in all four animals.

Hypophysectomy. The pituitary was removed by the parapharyngeal approach in a series of ten ovariectomized animals. Estrin and progesterone were then injected and the animals were observed for estrous behavior. In three, sexual responses occurred, while in the remaining seven repeated injections of the hormones never were followed by sexual behavior.

Examination of the sellae of these animals revealed that the pituitary had been completely removed in the seven animals in which no sexual behavior occurred. Moreover, fragments of the gland, the volume of which

was estimated to be one-fifth to one-third of that of the intact gland, were found in the animals in which sexual behavior occurred following the injections.

DISCUSSION. The experiments which have been described in the preceding sections demonstrate conclusively that ovulation and the subsequent



Figs. 7 and 8. Sections through the brain of a guinea pig in which the mammillary bodies and other midline structures had been removed by suction. Evading responses were normal after the operation. Thionin, $\times 6$.

Figs. 9 and 10. Sagittal sections through the sella turcica of a guinea pig in which the pituitary stalk had been sectioned and the superior cervical ganglia removed. Mating behavior, evading responses and ovulation were normal after the operations (see text). Hematoxylin and eosin, $\times 11$.

changes which occur during the reproductive cycle in the guinea pig can occur in the absence of any nervous pathways reaching the pituitary through the pituitary stalk or the cervical sympathetic system. This conclusion is in agreement with the observations of Greep (1936), who reported that ovulation and pregnancy occurred after reciprocal trans-

plantation of the pituitaries of young male and female rats. It is obvious that the pituitary stalk was completely severed as a result of this operative procedure. Furthermore, the failure of ovulation after transplantation of the pituitary gland, as reported by Schweitzer, Charipper and Haterius (1937), seems more reasonably explained by the assumption that there is an insufficient functioning of the gland in its abnormal graft site than by their assumption that the causative factor is the interruption of the nervous supply. That the gland is, indeed, abnormal after transplantation was shown by these authors and by Buxton (1936), who found that it was markedly basophilic and gradually underwent absorption. Likewise, in the present experiments, suppression of ovulation often occurred for long periods after cutting the pituitary stalk, a much less radical operative procedure than transplantation. It would seem, therefore, that in spontaneously ovulating species, as represented by the rat and the guinea pig, the reproductive cycle is under the control of some mechanism other than the nervous system.

In earlier experiments it was shown that ovulation in the guinea pig is not hastened by injections of estrin on the 12th to 15th days of the cycle (Dempsey, Hertz and Young, 1936) and that continuous injections of estrin do not destroy the inherent rhythm of the reproductive cycle (Dempsey, 1937). These facts have caused us to question, for the guinea pig, the validity of the current theories of the endocrine control of the reproductive cycle, as these theories depend largely upon the assumption that a rising concentration of estrin inhibits the production of follicle-stimulating hormone and causes the release of the luteinizing hormone from the pituitary. It should be noted, however, that these theories appear to explain satisfactorily the release of the luteinizing hormone in the rat. Moreover, in the abbreviated cycle of the rat it is doubtful if the corpora lutea are ever functional. In the guinea pig, on the contrary, with its longer cycle and its well-marked luteal phase, none of the existing theories of endocrine control of the cycle seem to explain the facts satisfactorily. The data presented in the present paper, likewise, are difficult to reconcile with the hypothesis that the cycle in the guinea pig is controlled through the nervous system as it apparently is in the rabbit, in which copulation or sexual excitement is a stimulus for ovulation and transection of the pituitary stalk abolishes this response (Brooks, 1937). It seems necessary, therefore, to postulate that in the guinea pig the cycle is under hormonal control but that there exists another endocrine factor which as yet has not been taken into account. In this connection it is significant that progesterone is known to inhibit the release of the luteinizing hormone (Makepeace, Weinstein and Friedman, 1937; Dempsey, 1937).

Data are presented in foregoing pages of this paper which indicate certain parallelisms between the sexual responses which occur when the animal

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is in heat and the evading reactions which occur during the diestrous interval. Both are caused by the same stimulus, rubbing the hair on the back and rump. After all lesions of the nervous system which leave the estrous responses intact, the evading responses are also intact. The converse is also true with the single exception that after complete hypophysectomy injections of estrin and progesterone no longer cause heat, while normal evading responses are obtained. After section of the pituitary stalk, however, heat and evading reactions both may be demonstrated, indicating that the failure of the sexual responses after hypophysectomy must be attributable either to a hormonal deficiency or to a non-specific effect on the general condition of the animal.

CONCLUSIONS

Normal reproductive cycles were observed in guinea pigs in which brain lesions had been produced in the septal and preoptic regions, the pretectal area, and after section of the pituitary stalk. Lesions destroying the inferior colliculi, while abolishing mating behavior and the evading responses to sexual stimuli shown during the diestrus, do not interfere with ovulation.

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THE AMERICAN JOURNAL OF PHYSIOLOGY

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THE AMERICAN PHYSIOLOGICAL SOCIETY

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